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Monoterpenes of Ponderosa Pine Xylem Resin in Western United States

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MONOTERPENES OF PONDEROSA PINE XYLEM RESIN IN WESTERN UNITED STATES

by

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The xylem resin of more than 5,700 *Pinus ponderosa* Laws. trees at 68 locations in Western United States was analyzed in a 9-year study. The quantity of the five major components— α -pinene, β -pinene, 3-carene, myrene, limonene—of the monoterpene portion of individual trees varied widely over the range of the species. This range can be conveniently divided into five regions and four transition zones on the basis of plot averages for the five components. A working genetic hypothesis is proposed for classifying individual tree types based on monoterpene composition; 115 types were found. One of the rare composition types, high in limonene, may prove to be resistant to attacks by the western pine beetle (*Dendroctonus brevicomis* Lec.), a major pest of ponderosa pine. Results are also reported on: quantity of resin flow; percent terpene in whole resin; nongenetic influence on qualitative composition; unusual amounts of minor monoterpene components; amount of sesquiterpene; association of monoterpene composition with resin color, bark color, and needles per fascicle; association of resin quantity and quality with bark beetle distribution, abundance and host resistance.

KEY WORDS: *Pinus ponderosa*, xylem resin, monoterpenes, variation, genetics, natural resistance, *Dendroctonus brevicomis*, α -pinene, β -pinene, 3-carene, myrcene, limonene, terpinolene, β -phellandrene, sabinene, gas chromatography.

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PRINTER'S ERROR

Through oversight, printer's identification lines at the top of pages 30 and 44-48 were not removed in the final press run of this publication. These lines are not part of the text.

INTRODUCTION

Ponderosa pine (*Pinus ponderosa* Laws.), one of the most valuable pines in the world, is widely distributed in North America. Much has been written about it: Axelton (1967) recorded some 2,200 references to ponderosa pine, and in possibly 40 percent of these, it is the primary subject. Fowells (1965) provided good general description of the tree and its characteristics.

The variability of ponderosa pine has commanded the attention of many observers. Variation in growth characteristics has been of most interest, and a wide range of performance has been attributed to seed source, climate, elevation, planting site, or the interaction of these and other conditions.

The variations in composition of the xylem resin—a super-saturation of resin acids in terpenes—in this species have been investigated also. Schorger (1919) first reported on differences in the proportions of the monoterpenes—one of the major groups of terpenes. This report stood unchallenged until Hagen-Smit and others (1950) found trees with a large percentage of 3-carene; Schorger had reported no 3-carene. Mirov (1961) then investigated the regional characteristics of ponderosa pine xylem terpenes and reported results in a series of papers. He found considerable regional variation and called 3-carene the indicative terpene of ponderosa pine. Mirov's and earlier reports were based on aggregate samples from several trees—a necessity in procedures then available. Variation between trees was therefore not detectable.

Commercially the resin acids are often called *rosin*; the terpenes, *turpentine*. The three groups of terpenes are: monoterpenes ($C_{10}H_{16}$), which are readily volatile and usually give resin its characteristic odor; sesquiterpenes ($C_{15}H_{24}$); and diterpenes ($C_{20}H_{32}$), which are larger molecules and are much less volatile.

With the development of gas chromatography, single-tree samples can be analyzed quickly. Pelouquin (1964), Smith (1964b), and Smith and others (1969) found large local variation in ponderosa pine terpenes. They also reported some trees without 3-carene. Smith (1964a, 1964c, 1968) found within-tree constancy in composition.

Of particular interest was the possible association of xylem resin of ponderosa pine with tree resistance to the western pine beetle (*Dendroctonus brevicomis* Lec.) and with beetle attraction to the tree. Quantity and quality of resin strongly influenced success of forced attacks (Smith 1965, 1966, 1969), and myrcene—one of the terpenes—synergized pheromones produced by the beetle (Bedard and others, 1969).

These findings—variation in growth characteristics of ponderosa pine, local and regional variation in its xylem monoterpenes, preliminary reports on within-tree constancy of terpenes, and the possible association of xylem monoterpenes with resistance and host specificity of bark beetles—suggested the need for an intensive and extensive analysis of the xylem resin of ponderosa pine in Western United States. This study sought to define regional types of ponderosa pine monoterpenes more accurately, locate zones of transition, gather information on the frequency and kinds of individual tree types of composition, possibly gather evidence on the genetics of monoterpenes from these data, and locate candidate trees for studies of resistance to bark beetle attack.

This report summarizes information from resin samples obtained at 68 locations representing the range of ponderosa pine in the United States. It proposes a regional type classification, and offers a genetic hypothesis for classifying individual tree types, based on the monoterpene composition of the xylem resin.

PROCEDURES

Plot Location

The regional type study used data collected from 5,165 trees in 68 plots, chosen to represent the range of the tree in the United States (fig. 1; table 1) but adjusted to sample more heavily those regions of unusual interest indicated by Smith and others (1969). The design called for plots of 80 trees each, to be made up of two 40-tree subplots 1 to 5 miles (ca. 1.6 to 8 km) apart. At a few locations, because of lack of time and the scattered tree distribution, a

single large plot was used. One day was allotted to sampling each plot. In only three plots was there more than 500 feet (ca. 150 m) difference in elevation between the two subplots. Of the actual plots used, the first 62 were full size, averaging 76 trees per plot. The remaining six plots included fewer than 40 trees. In four of these plots, the small number of trees was again due to lack of time and the very scattered distribution of trees in the stand. The other two were additional subplots within a few miles of a full-size plot.

Table 1.—Number, name, and location of plots for the study of ponderosa pine xylem monoterpenes in Western United States

Plot number and name	National Forest and State	Long. W	Lat. N
1 Lee Canyon	Toiyabe, Nev.	115° 39'	36° 21'
2 Hualapi Park	State Park, Ariz. ¹	113 53	35 06
3 Williams Mountain	Kaibab, Ariz.	112 11	35 13
4 Fool Hollow	Sitgreaves, Ariz.	110 04	34 15
5 Timber Mountain	Tonto, Ariz.	110 34	33 40
6 Mt. Graham	Coronado, Ariz.	109 49	32 39
7 Rustlers Park	Coronado, Ariz.	109 16	31 55
8 Bear Canyon	Coronado, Ariz.	110 42	32 21
9 Indian Springs	Prescott, Ariz.	112 31	34 28
10 Jacob Lake	Kaibab, Ariz.	112 15	36 45
11 Wheatfield	Navajo Reserve, ¹ Ariz.	109 08	36 13
12 Turkey Spring	Cibola, N. Mex.	108 31	35 25
13 Sulfur Canyon	Cibola, N. Mex.	105 41	35 07
14 Clear Creek	Santa Fe, N. Mex.	106 50	36 00
15 Black Canyon	Sante Fe, N. Mex.	105 52	35 44
16 Cloudcroft	Lincoln, N. Mex.	105 43	32 58
17 Cherry Creek	Gila, N. Mex.	108 13	32 55
18 Luna	Apache, N. Mex.	109 02	33 49
19 Tusayan	Kaibab, Ariz.	112 07	36 00
20 Idaho Springs	Arapaho, Colo.	105 32	39 45
21 Yellow Pine	Medicine Bow, Wyo.	105° 25'	41° 16'
22 Sheep Mountain	Medicine Bow, Wyo.	105 15	42 13
23 Jewell Cave	Black Hills, S. Dak.	103 57	43 43
24 Red Cloud	Nebraska, Nebr.	103 01	42 44
25 Mud Creek	Shasta-Trinity, Calif.	122 04	41 15
26 Big Trees	Sequoia, Calif.	119 02	36 44
27 Greenhorn	Sequoia, Calif.	118 34	35 45
28 Onion Valley	Inyo, Calif.	118 17	36 47
29 Tehachapi Park	County Park, ¹ Calif.	118 31	35 06
30 Figueroa Mountain	Los Padres, Calif.	119 59	34 45
31 San Martin	Los Padres, Calif.	121 26	35 54
32 Cold Spring	Los Padres, Calif.	121 41	36 13
33 Saw Mill Flat	Mendocino, Calif.	122 46	39 06

¹ Other than National Forest.

Table 1—*Continued*

<i>Plot number and name</i>	<i>National Forest and State</i>	<i>Long. W</i>		<i>Lat. N</i>	
34 Harrison Gulch	Shasta-Trinity, Calif.	122 °	56 '	40 °	22'
35 Mad River	Six Rivers, Calif.	123	29	40	26
36 Big Creek	Stanislaus, Calif.	120	06	37	49
37 Iron Mountain Road	Eldorado, Calif.	120	33	38	45
38 Bogard	Lassen, Calif.	121	06	40	36
39 Lee Summit	Plumas, Calif.	120	45	39	52
40 Adin Pass	Modoc, Calif.	120	55	41	21
41 Camas Creek	Fremont, Oreg.	120°	13'	42°	13'
42 Lehman Creek	Humbolt, Nev.	114	15	39	01
43 Charlton Flat	Angeles, Calif.	118	01	34	18
44 Blue Jay	San Bernardino, Calif.	117	13	34	14
45 Fuller Creek	San Bernardino, Calif.	116	44	33	48
46 Duck Creek	Dixie, Utah	112	41	37	31
47 Ponderosa	Fishlake, Utah	112	30	38	15
48 Woodland Park	Pike, Colo.	105	04	39	01
49 Cuchara	San Isabel, Colo.	105	07	37	22
50 Alder Creek	Rio Grande, Colo.	106	39	37	43
51 Junction Creek	San Juan, Colo.	107	55	37	20
52 Devils Canyon	Manti-LaSal, Utah	109	24	37	44
53 Lava Cast	Deschutes, Oreg.	121	23	43	52
54 Teiton-Willows	Snoqualmie, Wash.	120	01	46	41
55 Blue Creek Cut-off	Colville, Wash.	117	48	48	19
56 St. Regis	Lolo, Mont.	115	07	47	18
57 Twin Creek	Salmon, Idaho	113	58	45	37
58 Porcupine Creek	Helena, Mont.	112	16	46	35
59 Lincoln	Helena, Mont.	112	39	46	57
60 Smith River	Lewis & Clark, Mont.	111	08	46	45
61 Horseshoe Mountain	Big Horn, Wyo.	107	24	44	47
62 Green Lakes	Ashley, Utah	109°	36'	40°	51'
63 Middle Mountain	State Park, ¹ Calif.	116	35	33	58
64 Red Hole	Fishlake, Utah	111	27	38	45
65 Poncha Pass	San Isabel, Colo.	106	06	38	26
66 Current Creek	Humboldt, Nev.	115	21	38	48
67 Wheeler Slope	Humboldt, Nev.	114	16	39	01
68 Ranger Peak	Los Padres, Calif.	119	59	34	43

¹ Other than National Forest.

For supplementary studies of the relation of position and time to monoterpene variation, and for a high-limonene resin study, additional trees were sampled, as noted in the Results section.

Field work on the regional study began in spring 1968 and concluded in fall 1971. Field work on the supplementary studies extended from 1962 to 1971.

Sampling

Each plot was assigned 80 tree numbers running consecutively; numbers 1 through 80 were assigned to plot 1, 81 through 160 to plot 2, etc. Plot trees were selected along a line transect that best represented the area. This design prevented tapping a tree twice and made a given tree easier to relocate. Only trees of more than 10 inches (ca. 25 cm) d.b.h., and

thus more than 30 years old, were sampled; those with wounds or serious defects were excluded. (In a supplementary study of trees adjacent to one plot, the diameter limit was lowered to 5 inches [ca. 12.5 cm] d.b.h.)

As each tree was tapped, its diameter and bark color were noted. A cursory estimate of needles per fascicle for most of the stands was made by a quick count of a sample of needles from the ground under the stand. It should be emphasized that the count was for the stand and not for individual trees.

Each tree was tapped with a 9/16-inch (ca. 14.3 mm) bit and brace at a height of about 4 feet (ca. 1.2 m). (Trees for a supplementary study were tapped at several heights, as noted in the Results and Discussion section.) The hole was made at a slightly up-

ward angle through the bark and less than 1/2 inch (ca. 1.3 cm) into the xylem. The hole was cleared of debris, and a 5-cm³ collecting vial was fitted into the hole so that the lip was past the phloem and just into

the xylem. Before the hole was drilled, a small area of bark was usually shaved down to within 1/4 inch (ca. 0.6 cm) of the phloem to facilitate placement of the vial. The characteristic deep fissures in the bark,

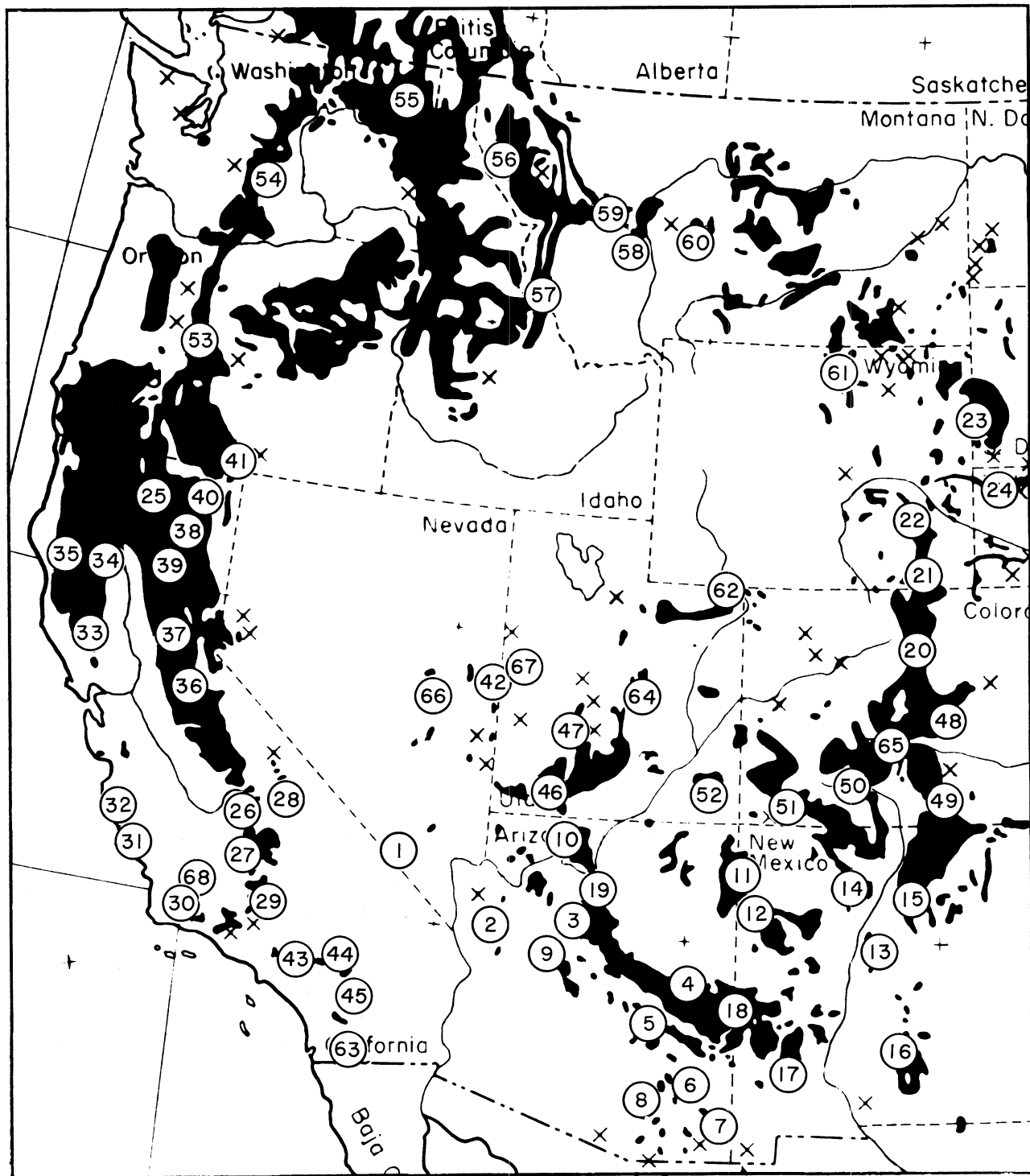


Figure 1.—Plots in Western United States used in the study of ponderosa pine xylem monoterpenes are shown on a map of the species' distribution; X=isolated occurrence (Critchfield and Little 1966).

particularly in older trees, were ideal locations for tapping.

Vials were removed 6 to 12 hours after the tap was set and were stoppered with a cork covered with aluminum foil. A #4 cork, with the tree's number, was placed firmly in the tap hole as a temporary mark. Generally, taps made in the morning or early afternoon were picked up late the same day; those made late in the afternoon, the next day.

Within 12 hours after vials were collected from a plot, an analytical preparation was made of the resin from each tree. Approximately 0.5 cm³ of resin was drawn from the collecting vial and transferred to a labeled, preweighed, 1/2-dr, screw-cap sample vial containing 0.7 g of pentane (chromatographic quality). The cap, with a Teflon¹ gasket, was tightly fastened and the vial agitated to dissolve resin sample. Each sample vial with its contents was reweighed in the laboratory. Collection vials and vials containing the analytical sample were kept tightly sealed and under refrigeration except when being processed.

If there was less than 0.4 cm³ of resin in the collecting vial, the pentane in the analytical sample vial was poured into the collecting vial to about equal the volume of resin and the remainder discarded. The resin was dissolved and pipetted back into the sample vial. This procedure produced a sample suitable for all analyses except weight.

At the time of each sample preparation, resin was classified as colorless or as one of six shades ranging from light amber to dark yellow. No reference charts classifying colors were used; all samples were classified by the same preparer, whose judgment and recollection was relied on.

Laboratory Analysis

All prepared samples were quantitatively and qualitatively analyzed for monoterpenes on a gas-liquid chromatograph with a thermal conductivity detector. Gas-liquid chromatography separates volatile substances by percolating a gas stream over a stationary liquid phase. This phase is thinly spread over fine solid particles and packed into a column. Different groups of molecules are separated by the selection of the stationary phase and the temperature. When the volatiles are passed over an electronic detector, an electric signal is produced and a

recording device can chart the responses graphically. The peaks in the chromatogram represent the components of the mixture. The quantity and quality of the volatiles can be interpreted from chromatograms. As the volatiles are eluted from the column, they can be recovered by collecting them in chilled containers, by a technique called preparative gas chromatography.

Each sample was analyzed on both a fast and a standard column (Smith and Greene 1971). Both columns had the same stationary phase of 5 percent β , β' oxydipropionitrile; the fast column was 5 feet (ca. 1.5 m) by 1/8 inch (ca. 3.2 mm) stainless steel, with 60/80 mesh Chromosorb W A/W; the standard column was 12 feet (ca. 3.7 m) by 1/8 inch (ca. 3.2 mm) stainless steel, with 80/100 mesh Chromosorb W A/W. Operating conditions for both columns were 135 to 145° C on the injector, 65 to 70° C on the column, 155 to 160° C on the detector, 45 to 55 ml of helium per minute at the outlet port. Sample size ranged from 0.6 to 1.0 μ l on the standard column and 0.1 to 0.2 μ l on the fast column.

Each sample was analyzed first on the fast column within 3 months after sample preparation. The solvent peak was attenuated to 125 or 250 to keep it on chart, but all monoterpene peaks were analyzed at an attenuation of 4. This analysis took about 3 minutes. The resulting chromatogram served as a guide to attenuation for the analysis on the standard column, as a visual check on calculations made on the standard chromatogram, and as a check on any possible change in the sample during storage.

Each analytical sample was run on the standard column within 8 months of preparation (most were run within 5 months). This analysis required 12 to 15 minutes, and the chromatogram was used for qualitative and quantitative calculations.

Qualitative determinations were made from the relative retention time to each peak, with α -pinene given a base value of 1.0. For convenience, a series of transparent acetate templates marked with the position of the other monoterpenes relative to changing values of α -pinene was prepared. When the proper template, selected for the elution time to α -pinene, was placed on the chromatogram, the identity of each peak could be read directly from the template without measuring or calculating relative retention times.

Quantitative calculations were made by normalizing disc integrator values of peak areas; that is, each monoterpene was calculated as a percentage of the total amount of monoterpenes.

¹ Trade names and commercial enterprises or products are mentioned solely for necessary information. No endorsement by the U.S. Department of Agriculture is implied.

The behavior of sesquiterpenes needs some explanation here. These pass along the chromatographic column at about one-third to one-fourth the speed of the monoterpenes. Because another sample usually was injected as soon as the monoterpenes of the previous sample had eluted, a sesquiterpene peak was separated from the monoterpene peaks of its sample by the monoterpene peaks of one or more other samples. No record was kept at this time of the sesquiterpene peaks, which were small; however, by properly timing the injection of samples, it was possible to work around them so that they did not interfere with the monoterpene analysis.

Two other columns with different stationary phases were used to verify the qualitative determinations of the standard columns; one was 10 percent LAC-446, and the other, 10 percent Apiezon-L. They were operated like the standard column except that the column temperature was 110° to 120°C. Three to five representative samples from 40 representative plots were run on these two columns.

Two additional qualitative verifications were made. For the first, an aggregate sample was made using a three- to five-drop aliquot from each of the first three samples of each plot. This aggregate was, in effect, a representative sample of ponderosa pine in Western United States. Individual peaks were isolated and recovered by preparative chromatography. These isolations were then verified on the β , β' and Apiezon columns. The assumption was that peak identification was correct for all trees if the individual peaks remained qualitatively and quantitatively valid on both columns after preparation. For the second verification, peaks were isolated and recovered from samples of individual trees which showed unusual composition, particularly in minor components, and were verified on the β , β' and Apiezon columns.

The percentage of monoterpenes in whole resin was determined from the resin remaining in the collecting vial after preparation of the analytical sample. Because yield was insufficient from many trees, only about 2,800 trees could be analyzed in this way. Two subsamples of each suitable resin sample were apportioned into small weighing vials and placed at 60° C. for 3 days and then at 80° C. until weight loss ceased. These subsamples ranged from 0.15 to 0.25 g; this was sufficient to insure that all samples had equal evaporative surface area. The percent loss of weight was calculated for the two subsamples and averaged for the sample. This was considered the percent monoterpene in whole resin

for the tree. Some samples were not analyzed for several months. Partial crystallization had started in many samples held more than 2 weeks; thus, all were thoroughly homogenized just before allotment to subsamples. A check was made of the effect of crystallization and homogenation on percent monoterpene for about 4 percent of all the samples. After the temperature regimen, the residue was dissolved in pentane and run on a fast column; the approximate proportions of monoterpene and sesquiterpene remaining in the sample were noted.

Statistical Analysis

To analyze the data, four computer programs were developed: (1) calculation of means of percent composition for each component for each plot; (2) conversion of percent to arc sine and calculation of means and upper and lower levels for 95 percent confidence for components by plots; (3) plotting of frequency distribution of percentages for each component for each plot; (4) conversion of intervals of percent-composition range to simple coded categories for determining resin types for individual trees. Arc sine values were used for plot analysis; unconverted values, for tree analysis.

As most of the distributions are normal or seem to be derivatives of the normal, the t-test would appear to be most applicable, although some distributions were bimodal or heavily skewed. Also, a conservative criterion of significance was adopted so that differences classed as significant are quite confidently so.

Checks on Analysis

Sample Size

In the supplementary study of high-limonene trees, data suitable for analysis of plot size were gathered and served as a check. Plot 40 was enlarged by 624 trees (plot 40A). All 624 trees were analyzed chromatographically on the short column; the first 80 were analyzed on the long column, along with every sixth tree of the remainder, for an additional 80 trees. These two sets of 80 trees, and plot 40 itself, have much the same frequency distribution, but certain extreme values do not occur in all three. Thus, an 80-tree sample may not detect certain kinds of trees that occur infrequently. The means for the three samples, however, show only slight and generally insignificant differences: one sample differed from the second in 3-carene and

from the third in β -pinene, and the second differed from the third in limonene (table 2).

A comparison of the 80 trees and the additional 624 shows that the two sets have essentially the same distribution and means, but, again, the primary plot does not have certain individual types of trees (appendix I).

Table 2.—Average normalized monoterpene composition for three 80-tree resin samples from a single stand (plots 40 and 40A, Modoc National Forest, Calif.)

Monoterpene	Sample		
	1	2	3
	Normalized composition ¹		
	Percent		
α -pinene	6.2	7.2	6.9
β -pinene	20.5 a ²	23.9	26.1 a
3-carene	44.3 b	37.2 b	42.1
Myrcene	13.8	14.3	12.2
Limonene	11.8	14.3 c	9.3 c
Terpinolene	2.6	2.2	2.5

¹Includes small amounts of minor components not listed.

²Values followed by the same letter differ significantly at the 5 percent level of probability according to t-test.

The study of plot size shows that the 80-tree sample was adequate for area analysis, but in certain locations it may not be sufficient for determining the individual types of trees in the area, especially in transition zones.

Chromatographic Analysis

All checks on qualitative determination on the LAC and Apiezon columns were positive, including

the individual peaks from the aggregate sample and from samples with unusual amounts of the minor components. Thus a high degree of confidence was established that components were correctly identified. Added to this assurance were numerous similar checks of ponderosa pine terpenes with known pure chemicals in earlier studies.

The stationary phase, β , β' oxydipropionitrile, is excellent for separating monoterpenes, but it does deteriorate with heat and use; 14 standard columns were used during the analysis. Inter-column variation in quantitative and qualitative measurements was virtually nonexistent, however. This is best illustrated by an analysis on the same standard column of one sample from each of 58 plots 1 to 3 years after they were analyzed on different standard columns (table 3). Correlations between the two sets of measurements exceeded 0.99; slope ranged only from 1.01 to 0.99.

Percent Terpene Determinations

Checks on the monoterpenes remaining in samples analyzed for percent terpene were consistent in showing that only trace amounts were present after the temperature regimen; the amount of sesquiterpenes was largely unchanged. The crystallization and homogenation of the samples of whole resin had no effect on percent terpene. Thus the procedure used to determine percent monoterpene in whole resin is accurate. It was used in preference to chromatographic procedures using proportional weights of resin and solvent, and of monoterpene and solvent peak counts. The large attenuation of the solvent peak necessary for the chromatographic analysis probably introduced greater error than that introduced by possible slight loss of monoterpenes with the temperature regimen. Also, many of the

Table 3.—Correlation of chromatographic analyses of average monoterpene composition on different standard columns: First analysis on various columns, second analysis all on another column 1 to 3 years later

Monoterpene component	Normalized composition ¹		Samples	Correlation coefficient
	First analysis	Second analysis		
	Percent			
			No.	(r)
α -pinene	22.0	21.7	58	0.99
β -pinene	18.9	18.7	58	.99
3-carene	43.5	43.2	51	.99
Myrcene	7.0	7.0	58	.99
Limonene	6.2	6.3	55	.99

¹Includes small amounts of minor components not listed.

analytical samples had developed varying degrees of crystallization between preparation and analysis, and this changed the weight ratios of resin to solvent. This partial crystallization did not change the normalized monoterpene composition.

No evidence was found in this or other studies of ponderosa pine resin of any change in the monoterpenes in a sample over a period of refrigerated storage as long as three years.

RESULTS AND DISCUSSION

The primary result of the study is information leading to a more precise and inclusive classification of regional types of monoterpene composition. A genetic hypothesis has also been developed, and a classification of individual tree composition types is based on it. In addition, general information has been gathered, including measurements of resin flow quantity, percent terpene, and distribution of the influence, if any, of site and of time and within-tree point of sampling. This general information will be reviewed first, as background for the classifications. Results of observations on minor components, resin color, and certain tree characteristics then follow, concluding with a discussion of the probable relation of monoterpene composition to bark beetle attack.

Resin Flow

The quantity of resin flow from trees varied widely. Because trees were tapped under an equally wide range of sites and conditions, no reliable statement can be made about regional characteristics. All plots had trees with good flow and trees with poor flow. Most plots had less than five trees without any flow over the period of tapping; a few plots had from 10 to 15. Reduced flow could usually, but not always, be attributed to cold weather. Under warmer conditions these trees would very likely have produced resin. For example, in plots 1 and 29, the only ones that had to be resampled, trees were tapped first early in spring, with snow still on the ground; about half failed to yield resin. When retapped in midsummer, virtually all trees yielded resin. Trees that yielded resin at the earlier tapping were generally the better yielders at the second tapping, however.

Usually the trees showing better yield were of middle-age and could be classified as vigorous; those of poorer yield were often suppressed or overmature. There were many striking exceptions, however, with poor yield from apparently vigorous trees and good yield from trees of apparently poor vigor. It was not unusual in many plots for some trees to yield 5 cm³ of resin in 1 or 2 hours, whereas adjacent trees

yielded nothing during this period and less than 0.5 cm³ in 6 to 8 hours. Often both types of trees could be visually classed as equally vigorous. As many as 5 to 10 percent of the trees had unusually high rate of resin flow. Such trees might be considered quite resistant to bark beetle attack (Mason 1966, Smith 1972, Wood 1962).^{2,3}

It is notable that despite considerable variation in the tapping conditions and in the flow time allowed, there was some flow of resin from about 95 percent of the 5,165 trees in the 68 plots; the flow from 5 to 10 percent of these trees was very slight, though, generally less than 0.1 cm³. Trees with little or no flow of resin could be considered highly susceptible to bark beetle attack (Mason 1966, Smith 1972, Wood 1962).^{2,3}

Percent Terpene

Samples from 2,800 trees were suitable for determining the percent of terpene in whole resin by the temperature-regimen and loss-of-weight procedure. Most of the 65 plots which were used averaged between 24 and 26 percent, though a few averaged about 22 percent, and a few 28 percent; the range for individual trees was from 15 to 35 percent (fig. 2; appendix II). Ninety-six percent of the individual tree samples fell between 19 and 29 percent, and 66 percent of the samples between 22 and 26 percent. Samples from some of the plots tapped early in the study were not analyzed for several months. These plots have a slightly lower average, and there might have been a small loss of total volatiles during the long storage. Plots sampled later, and analyzed after a much shorter storage time, show less variation between and within plots. The longer storage period did not effect the monoterpene analysis, since the

² Mirov, N.T. 1928. A study of western yellow pine (*Pinus ponderosa*) as the host tree of *Dendroctonus brevicornis*. Unpublished report. [On file at Pac. Southwest For. and Range Exp. Stn. Berkeley, Calif.]

³ Callahan, R.Z. 1955. Oleoresin production in the resistance of ponderosa pine to bark beetles. Unpublished report. [On file at Pac. Southwest For. and Range Exp. Stn., Berkeley, Calif.]

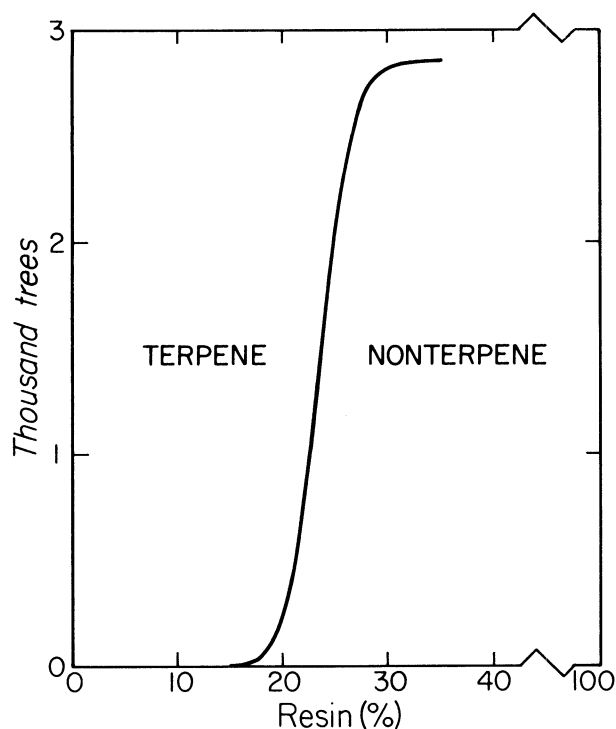


Figure 2.—The percent terpene in the xylem resin of 2,800 ponderosa pines ranked cumulatively from 15 to 35 percent.

sample for analysis had been taken from the collection vial within 24 hours after tapping the tree.

Samples from a set of 223 trees were classed for rate of flow, to see if it was associated with percent terpene. The set was divided into three generalized categories of low, medium, and high rate of flow. Only a slightly higher percent terpene was found in the group with the greatest flow rate:

<u>Yield class</u>	<u>Trees in class</u>	<u>Percent terpene</u>
Small	84	24.0
Medium	62	24.2
Large	77	25.6

A few samples taken at different vertical positions within a tree were suitable for percent terpene determination. These showed a difference no greater than 2 percent between various positions.

A general inspection of percent terpene from a wide range of regional monoterpene types shows no correlation between the two. The only suggestion of an association is between high α -pinene trees in region V and the higher percent terpene. In several plots, however, individual trees of the high α -pinene type do not show such a relationship, and trees with low α -pinene percent have a percent terpene equal to those with high α -pinene.

These results agree with most reports on percent

terpene. Mirov (1961) reports an average of 20 percent for 12 sources of ponderosa pine; the range was 17 to 27 percent. As his data were obtained from an aggregate sample from several trees from each source, they represent plot rather than individual-tree values. Samples from three to five plots from each of 12 areas selected to represent Mirov's locations gave an average of 24 percent, with a range of 22 to 27 percent. Williams and Bannister (1962) and Smith (1963) report 24 and 25 percent, respectively, for ponderosa pine. Mirov's lower values could be explained by the openfaced tapping procedure used, which resembled commercial practice. Though Zavarin and Cobb (1970) report a comparable average of 24 percent for a plot of 139 ponderosa pines in the central Sierra Nevada, the range of 13 to 37 percent is much greater than that reported elsewhere.

A comparison of the percent terpene of ponderosa pine with that of other pines shows considerable similarity. Mirov's data for 6 different pine species or varieties gives an average of 22.5 percent, with a range of 15 to 34 percent. Reports by Williams and Bannister (1962) and Smith (1963) for other species show a 2 to 4 percent higher value than Mirov's for the same species. Again, the differences may be due to collection method.

The narrow range of percent terpene would seem advantageous to the tree because of its relation to viscosity. One might speculate that if the amount were appreciably smaller, resin would not flow, whereas if it were much greater, resin would flow too quickly.

Major Monoterpene Components

The frequency distribution of percentage of the five major monoterpene components— α -pinene, β -pinene, 3-carene, myrcene, limonene—by plots reveals a wide range within and between plots and between components (fig. 3). Many distributions tend toward normal or normal with some degree of skewness; others tend to be bimodal or trimodal. Each of the components has a narrow distribution in some plots and a broad one in others. The range in all sampled trees for α -pinene is 1 to 99 percent; β -pinene, 0 to 72 percent; 3-carene, 0 to 88 percent; myrcene, 1 to 51 percent; and limonene, 0 to 70 percent. The breadth and configuration of distribution of each component is characteristic of a particular portion of the tree's geographical range; this might be interpreted as indicating differences in the frequency of alleles for each terpene.

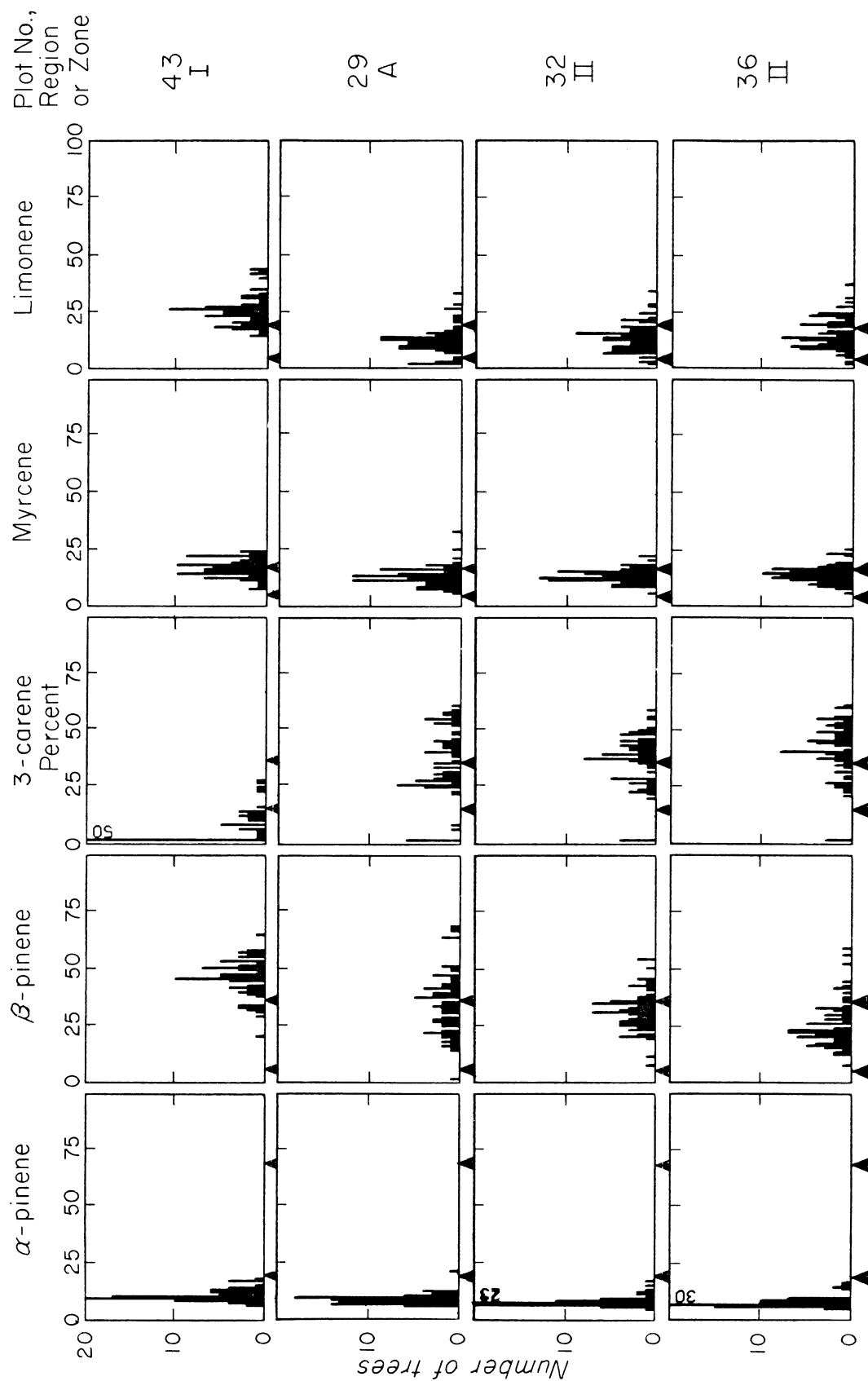


Figure 3.—Frequency distribution of the percentage of the five major monoterpene components in the ponderosa pine trees in each of 12 plots selected to represent each region and zone in the study. A 2-digit number within a box is the frequency for the indicated percentage when greater than 13. The two marks along the baseline for each component set off the three general percentage levels found for each component. (See figs. 1 and 6 for location of plots, regions and zones.)—Continued on p. 12

A, Plots 43-I, 29-A, 32-II, and 36-II.

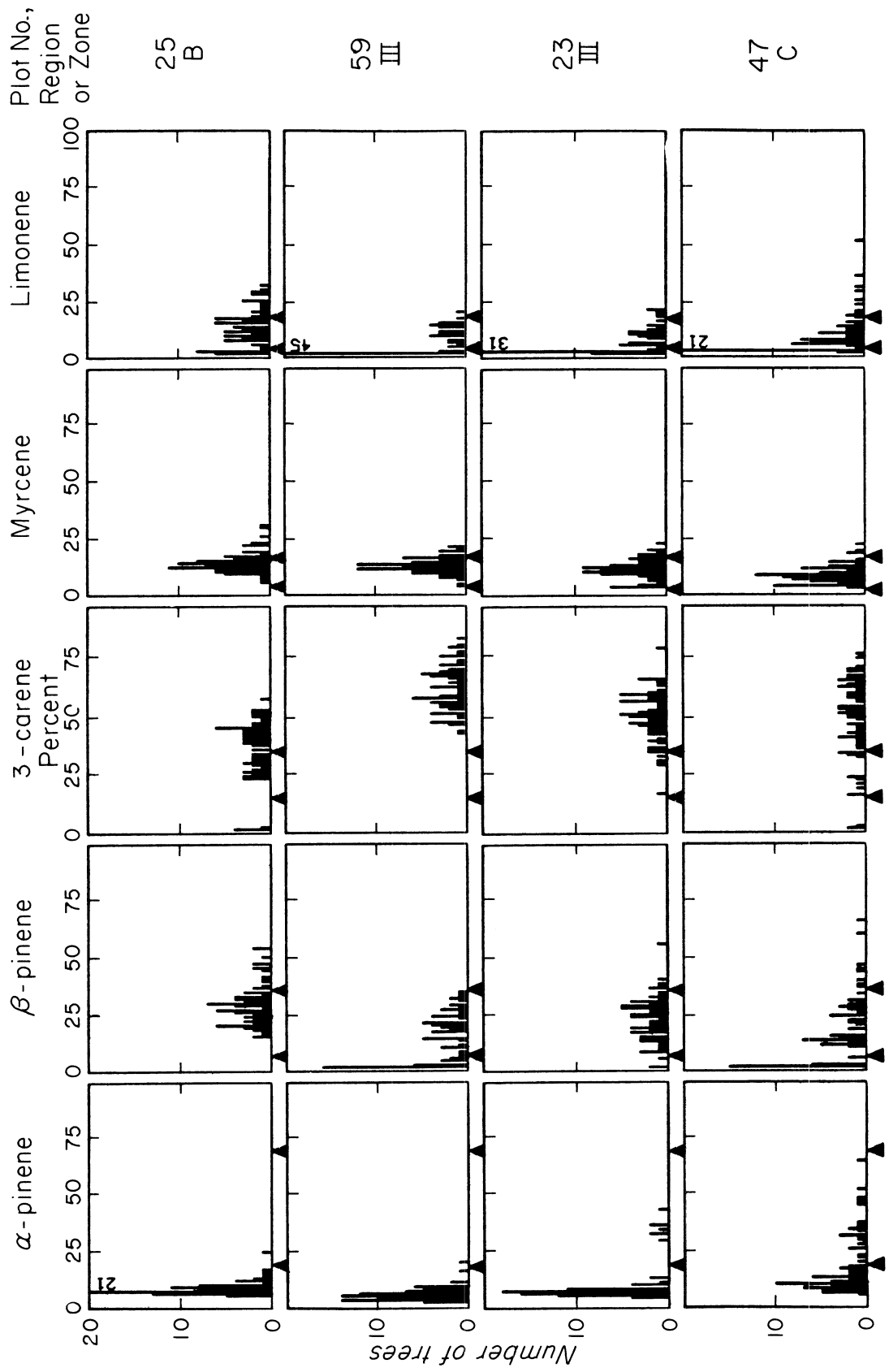


Figure 3.—Continued from p. 11. B, Plots 25-B, 59-III, 23-III, and 47-C.

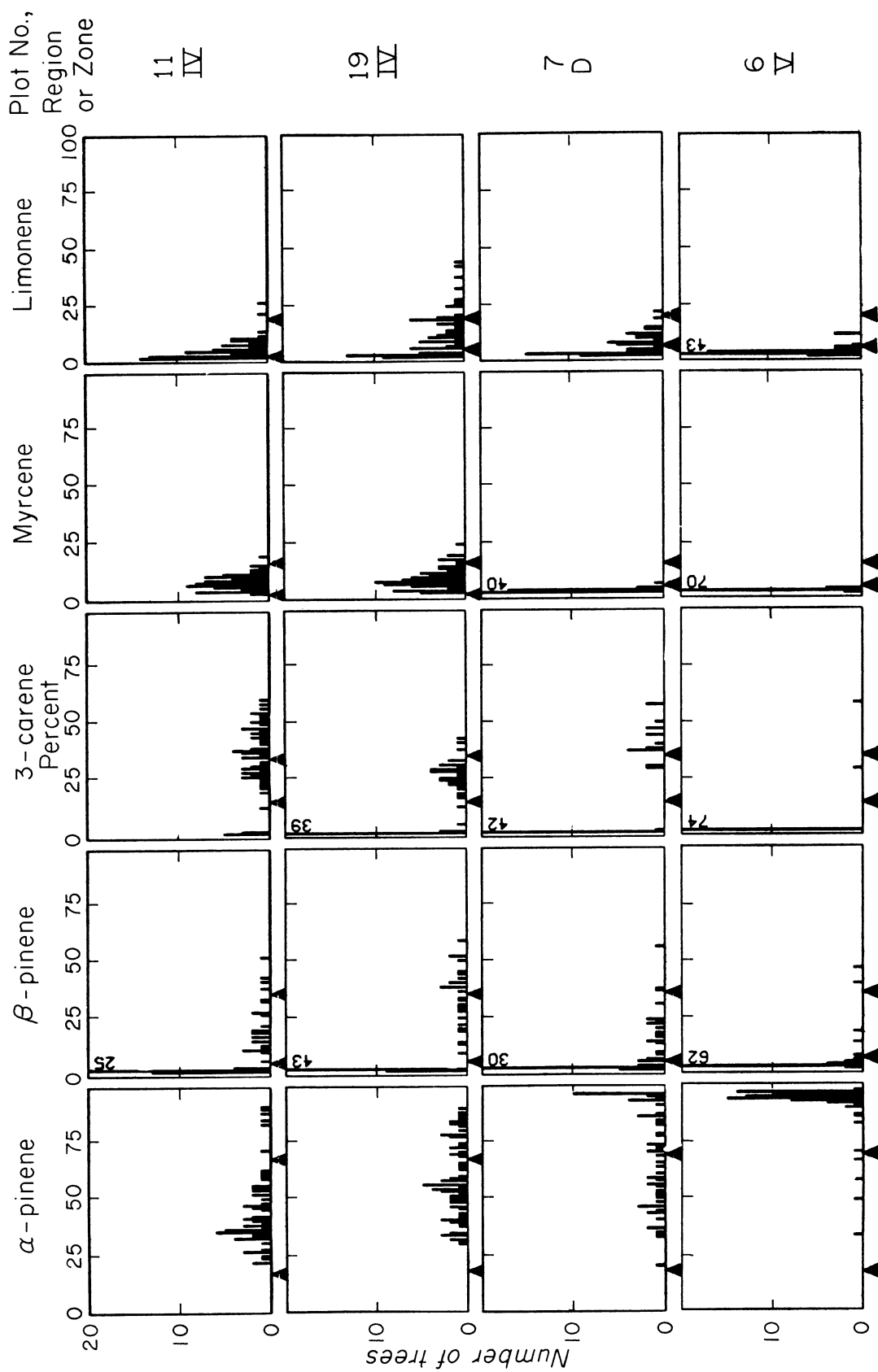


Figure 3.—Continued from p. 12. C, Plots 11-IV, 19-IV, 7-D, and 6-V.

Possible Nongenetic Influences

The data gathered indicate that monoterpene composition is nearly independent of place of growth of the tree and of time and within-tree position of sampling. Those changes that do occur might be considered fairly tight oscillations about a mean figure representative of the tree.

Effect of Site

The possibility that place of growth of a tree may have a measurable effect on resin composition has been considered. Forde and Blight (1964) found the xylem monoterpenes of planted Bishop pine, *Pinus muricata* (D. Don), to be strikingly similar to those of the naturally growing source trees, but very large

Table 4.—Average normalized monoterpene composition of paired plots of naturally growing ponderosa pine from this study (numbered) and of relatively corresponding seed sources (named, Smith and others 1969) growing at Placerville, Calif.

Plot	Trees	Normalized monoterpene				
		α pinene	β pinene	3-carene	Myrcene	Limonene
	Number	Percent				
3	75	50.2	4.2	26.9	4.3	11.2
Coconino	72	52.8	10.9	25.6	3.0	6.0
7	65	69.9	6.9	12.0	1.4	4.6
Chiricahua	12	62.1	9.9	21.9	1.8	1.7
22	75	6.2	22.1	53.2	9.6	5.7
Niobrara	9	7.0	34.2	46.0	4.2	3.0
23	70	8.5	21.9	50.8	10.1	4.5
Custer	7	5.7	32.5	44.6	8.4	6.3
24	72	9.0	22.1	48.9	11.0	4.4
Keyapaha	11	6.4	30.8	46.9	12.1	1.0
26	74	4.7	21.7	43.3	12.2	14.4
Tulare	5	5.4	23.5	46.7	9.8	10.0
27	74	4.7	22.2	43.4	11.6	14.4
Kern	11	7.5	27.3	43.2	8.2	9.6
33	80	6.8	32.1	33.3	13.4	11.3
Mendocino	8	11.8	45.9	21.1	10.2	8.9
36	80	5.8	24.2	40.2	12.6	13.8
Tuolumne	33	6.8	30.0	35.0	10.0	15.8
37	75	6.1	28.3	34.5	13.1	14.9
Eldorado	19	6.5	26.1	40.8	11.6	12.4
38	77	6.3	17.4	54.9	13.2	4.3
Lassen	28	7.2	25.9	47.9	10.9	6.8
41	80	5.2	13.5	56.2	13.2	4.2
Modoc	48	9.6	25.2	45.8	9.9	8.7
43	80	9.4	45.3	4.1	15.4	24.3
Los Angeles	5	12.9	42.8	10.5	14.5	17.2
44	74	9.1	46.2	4.2	13.7	24.8
San Bernardino	12	10.6	51.4	2.8	10.6	24.3
45	80	9.7	46.2	0.0	13.0	29.6
Riverside	12	10.6	55.3	0.0	10.6	22.8
48	80	4.4	14.3	67.1	6.4	3.3
El Paso	20	11.5	18.5	55.8	3.8	6.0
53	80	5.0	15.8	61.7	11.0	2.5
Deschutes	4	6.9	25.9	51.9	11.6	0.2
54	80	4.8	11.8	59.5	13.6	6.5
Pierce	6	7.2	10.0	60.4	11.6	7.0
57	76	4.8	13.0	59.2	12.4	6.8
Bitterroot	11	6.4	6.8	62.7	11.2	8.9

differences were attributable to source. Samples from trees in clones of western white pine, *P. monticola* (Dougl.), showed very little within-clone variation when the trees were grown on different sites, but the clones, regardless of site, were quite different from each other in resin composition (Hanover 1971).

Some of the plots in this study were suitable for a determination of the effect of place of growth, since they are in the general vicinity of the sources of seed used to establish a plantation at the Institute of Forest Genetics near Placerville, Calif., in 1929. The purpose of that plantation was to provide a common garden for studying regional differences in ponderosa pine. The monoterpenes analysis of the trees in this plantation was made in 1965 and 1966 (Smith and others 1969). Many of the seed sources in the plantation are represented by only one to five trees. And there were as few as 5 to 10 trees available for analysis of resin for some sources; whereas the plots used in this study usually had 75 to 80 trees. Additionally, the trees used as the seed source for the plantation were not the same trees as those used in this study. Despite these weaknesses, these were the best data available for comparing the monoterpenes of trees growing on their natural site with that of fairly comparable trees growing on a common site far removed from the seed source (table 4). The trees at the Institute were planted in 1929 and, therefore, fall within the size and age requirements of the over-all study. A regression analysis of these data shows a surprisingly good correlation, greater than 0.90 (table 5). These data support the assumption that place of growth of ponderosa pine has little effect on the composition of the xylem monoterpenes.

Within-tree position and time

In monoterpenes of various coniferous resin systems, both constancy and variation in composition have been found in samples from a single tree. A preliminary study showed that the xylem monoterpenes of ponderosa pine remained substantially the same when samples were taken from various vertical and horizontal positions and when samples were taken at different times (Smith 1964c). These results were substantiated by a more intensive study of radial position (Smith 1964a) and vertical and horizontal position (Smith 1968). Noticeable seasonal variation was found in the xylem monoterpenes of some Douglas-firs (*Pseudotsuga menziesii* [Mirb.] Franco), but some showed no variation (Hanover and Furniss 1966). Slight and insignificant variation was found in the xylem monoterpenes of Monterey pine (*Pinus radiata* D. Don) according to year and position of sampling (Bannister *et al.* 1962). Roberts (1970) found the xylem monoterpenes of slash pine (*P. elliotii* Engelm.) to vary greatly with vertical position in trees high in β -phellandrene but not in trees low in this monoterpene. This change was greatest near the base of the tree; all trees were being worked, or had been worked, for naval stores.

Possible variation with change in position or time is a critical element in use of monoterpenes in genetic research, plant classification studies, or work on the biological functions of terpenes. Therefore, two studies were made to determine the effect on resin composition of time and within-tree position of sampling.

Comparisons of samples taken at three points on 34 trees show, as reported previously (Smith 1968),

Table 5.—Correlation of monoterpene composition of resin samples from ponderosa pine in 19 native stands with samples from trees grown from seed collected within 1 to 50 miles of the native stand

Monoterpene component	Average normalized composition ¹			Correlation coefficient
	Native trees	Planted trees ²	Plots	
	Percent		Number	(r)
α -pinene	12.1	13.4	19	0.99
β -pinene	22.5	28.0	19	.91
3-carene	41.9	39.4	18 ³	.94
Myrcene	11.1	9.2	19	.90
Limonene	10.8	9.3	19	.91

¹Includes small amounts of minor components not listed.

²Institute of Forest Genetics, Placerville, Calif.

³Plot 45 had no measurable amount of a 3-carene.

that β -pinene increases slightly toward the top of the tree and limonene decreases slightly (fig. 4). In tendency, α -pinene was similar to β -pinene and myrcene was similar to limonene, whereas, 3-carene changed very little. Sets of comparisons show strong correlations, though base to middle is stronger than base to top (table 6). Three of the trees had appreciable changes with vertical position, however (fig. 4).

Ten of the 34 trees, including the three having the greatest change at the original sampling, were re-sampled at approximately the same vertical position 6 years later. Only trees 6 and 7 showed any appre-

ciable change over the 6-year period (table 7); these are two of the trees showing noticeable effect of vertical position at the first measurement.

The study generally supports the assumption that vertical position has a small but fairly consistent effect on monoterpene composition in ponderosa pine. Infrequently, however, a tree can be found that exaggerates this pattern or shows noticeable shift with time. The study also shows that a component lacking at the base of the tree was lacking elsewhere in the tree. Similarly, position of sampling did not influence the amount of a component that was present. Neither is it possible to associate monoterpene

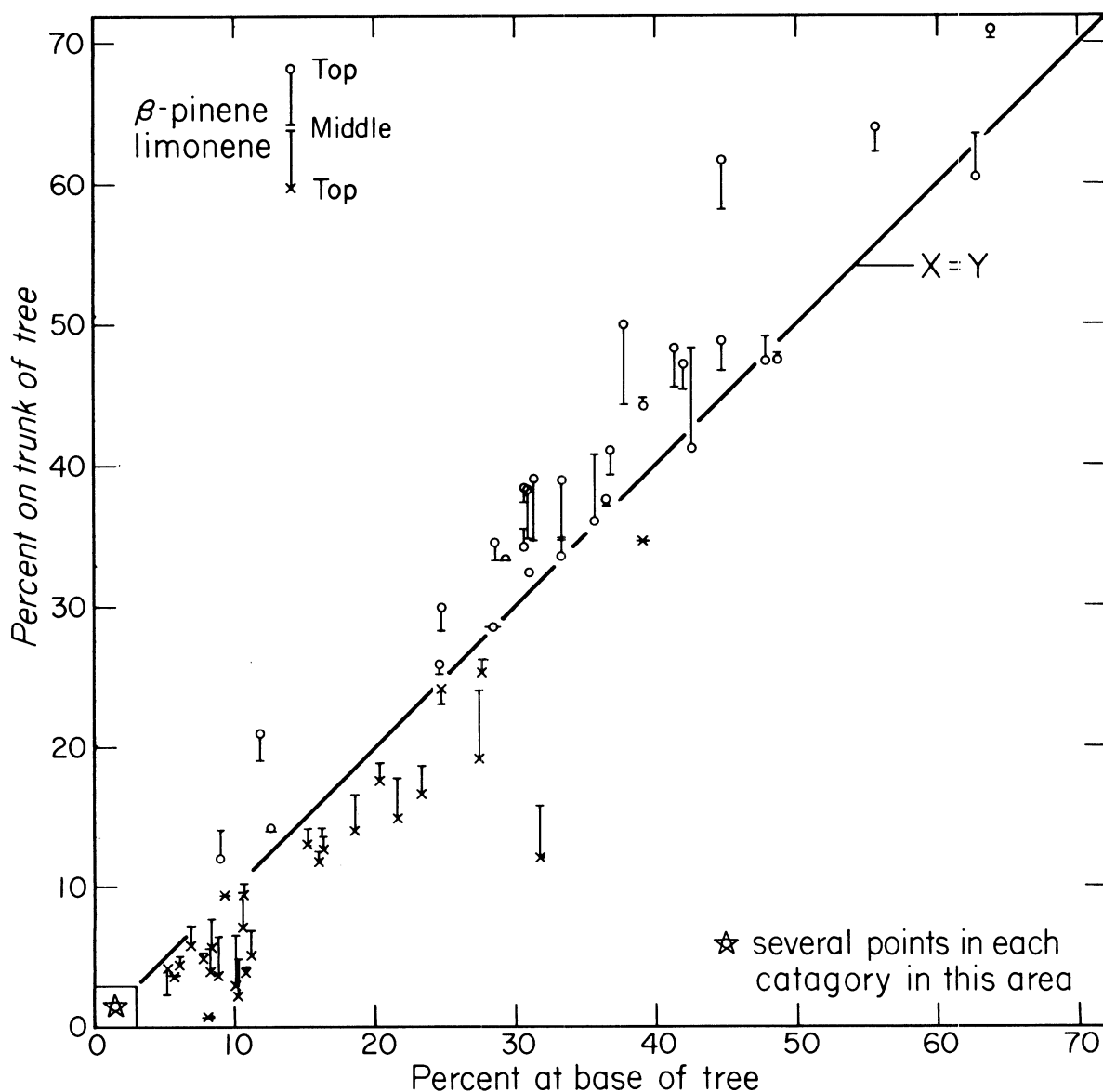


Figure 4.—Comparisons of percentage of β -pinene and limonene in resin samples from three positions on trunks of 34 trees showing only slight change with height.

composition and within-tree constancy. The tree with the greatest within-tree variation had nearly the same composition at the base as the tree with least variation.

Eight forked trees were examined, with the thought that differences might occur between forks but not within a single stem. Forked ponderosa pines occur infrequently and the two forks tend to be of about equal size. Samples were taken on the single stem of the tree at the base and on each fork at two or more comparable heights. As a fortunate coincidence, a wide range of monoterpene composition was found in the set of eight trees.

Regression analysis gave exceptionally strong correlations between forks, with values greater than 0.99. Correlations between tree base and top of one fork were very similar to those for the single-stem data for 34 trees discussed above.

Four sets of trees were used in a time study. They

were plot 29 with a 2-year interval; plot 1 with a 3-year interval; trees in a 40-year-old plantation at the Institute of Forest Genetics, Placerville, Calif., with a 7-year interval; and native trees at the Institute and on nearby Eldorado National Forest, with a 9-year interval. At the first tapping of plot 29 (Tehachapi Park) in 1969 and of plot 1 (Lee Canyon) in 1968, only 37 and 39 trees respectively ran resin because of cold weather. At the second tapping of both plots in 1971, resin was obtained for all 80 trees in each plot; however, only those sampled both times were suitable for the study of the effect of time.

Analysis of the data shows a very strong correlation of the sets of measurement, with *r* values all greater than 0.96 and most greater than 0.98 (table 8). Thus little or no change in composition with time occurred at the tree base during the 2- to 9-year study.

Table 6.—Correlation of average normalized composition¹ of monoterpenes in base-of-tree samples and in middle- and top-of-tree samples, ponderosa pine

Component	Trees	<u>Base-to-middle</u>		Correlation coefficient	Trees	<u>Base-to-top</u>		Correlation coefficient
		Base	Middle			Base	Top	
		<u>Percent</u>				<u>Percent</u>		
α -pinene	34	16.2	17.7	.99	33	16.6	19.3	0.99
β -pinene	33	27.9	30.8	.99	33	27.9	31.3	.98
3-carene	26	33.3	31.3	.98	26	33.3	30.8	.94
Myrcene	33	8.3	7.3	.93	33	8.3	6.6	.88
Limonene	33	12.3	10.0	.96	33	12.3	8.0	.89

¹Includes small amounts of minor components not listed.

Table 7.—Normalized monoterpene composition¹ of two ponderosa pines, out of 34 studied, with unusually large difference between measurements at the base and top trunk at two different years, Placerville, Calif.

	Tree No. 6			Tree No. 7		
Component	Base ²	Top	1971	Base	Top	1971
	1963			1963		
	Percent					
α -pinene	12	17	14	5	8	7
β -pinene	51	63	57	34	44	39
3-carene	0	0	0	41	29	36
Myrcene	9	7	9	7	6	5
Limonene	28	12	18	9	10	8

¹Includes small amounts of minor components not listed.

²Basal measurements for 1963 and 1971 were essentially alike.

Table 8.—Correlation of the normalized monoterpene composition of sets of ponderosa pines sampled at different years, by plots, number of trees, and sampling dates

Plot, number of trees, two sampling dates		α pinene	β pinene	Component 3-carene	Myrcene	Limonene
		Percent ¹				
Tehachapi Park, Calif.						
(37):	1969	7.3	36.0	35.2	12.7	8.5
	1971	7.4	35.9	35.3	12.6	8.4
	r	.99	.99	.99	.97	.99
Lee Canyon, Nev.						
(39):	1968	17.8	29.5	39.7	7.9	4.2
	1971	18.1	29.6	40.0	7.7	3.8
	r	.99	.99	.99	.98	.98
Placerville, Calif.						
(37):	1964	13.6	25.0	38.6	7.9	12.9
	1971	13.1	23.4	39.3	9.0	13.8
	r	.99	.99	.98	.96	.98
Eldorado National Forest, Calif.						
(37):	1962	12.3	31.9	33.9	9.7	11.0
	1971	12.7	31.3	33.2	10.1	11.1
	r	.99	.99	.98	.98	.96

¹Includes small amounts of minor components not listed.

Regions and Transition Zones

From the analysis of resin samples from all plots (fig. 5, appendix III) the range of ponderosa pine can be conveniently divided into five regions and four transition zones. The classification is based on similarities in xylem monoterpene composition between plots in regions and differences between plots in zones. Thus, plots in a region generally are similar to each other in average composition; plots in a zone generally differ from each other and from plots in adjoining regions in average composition.

The five regions are (fig. 6):

- I San Jacinto
- II Sierra-Pacific
- III Cascade-Northern
- IV Mogollon
- V Coronado

The four zones are:

- A Tehachapi
- B Modoc-Siskiyou
- C Escalante-Rio Grande
- D Chiricahua

Each has been named for a prominent geographical feature selected to avoid names used in other classifications of this tree. This classification is based

on plot averages and not on individual trees; tree variations will be discussed later in the report. Like all artificial classifications of biological phenomena, it has weaknesses, inconsistencies, and is open to individual interpretation. Nevertheless, there is considerable justification in the data for the plan proposed, although from 10 to 20 carefully chosen additional plots would help to refine the classification considerably.

Monoterpene composition differs significantly from one region to another in at least four components and often five (fig. 7). The monoterpene frequency distributions of a typical plot from each region (fig. 4) clearly show the sharp differences in composition, as well as the presence of some overlap in most components. Within each of the first four regions there are only small shifts in composition between plots. These are usually not significant, and a plot that represents the region can be selected. The fifth region, Coronado, is complex, with characteristics of both a region and a zone, although a typical plot has been selected. Within each of the first three transition zones, differences between plots are large and often significant, and a typical plot cannot be selected. The fourth zone, Chiricahua, consists of only one plot. The regions and zones vary widely in

extent, from more than 1,500 miles [ca. 2400 km] (III, Cascade-Northern) to less than 100 miles [ca. 160 km] (A, Tehachapi).

The validity of the regional and zonal divisions was checked by a plot-to-plot statistical traverse, noting significant changes in composition between two successive plots (fig. 8). Plots within regions differ by an average of 0.8 component, within zones by 2.8, and between regions and zones by 4.4.

To test the validity of using the 95 percent confidence level and aggregate plot data in determining boundaries, a comparison was made of within- and between-plot variation for the 62 full plots. Where two subplots had not been established, the plot was arbitrarily subdivided at the 40th tree. Difference between the two 40-tree subplots was significant in five plots in one component and in two plots in two components. Thus in 310 within-plot comparisons, only nine differences were detected. In 310 comparisons between plots, using the traverse data of figure 8 (plus the data obtained in comparing each zone with the adjoining regions), 107 differences were found. Plot means are therefore used with reasonable confidence that differences between plots indicate real and major changes in monoterpene composition.

Regions and zones are discussed here in a clockwise traverse of the tree's range from southern California northward through the Sierra Nevada and Cascades, then across the northern tier of States, and southward along the Rockies to southern Arizona (fig. 8). All plots except 66, 67, 68, which are too small or which are subplots near a standard plot, are included in the traverse.

I. San Jacinto Region

The first region—San Jacinto—encompasses the mountains of southern California. Plot data typically show a lack of 3-carene, with large amounts of β -pinene and limonene (fig. 6). A minor shift occurs at the San Geronio gap between plots 44 and 45; 3-carene is absent south of this line in plots 45 and 63, but is found in small amounts in plots 43 and 44 to the north and west of this line in the San Bernardino and San Gabriel Mountains. The amount of 3-carene increases northwesterly, but the rate of change is not sufficient to warrant including this area in a transition zone. A shift from the basic composition type to the south seems a better designation. This region terminates at the Tehachapi transition zone.

A. Tehachapi Transition Zone

In the Tehachapi transition zone the amount of 3-carene increases rapidly, but more so northward than westward. The increase in 3-carene is balanced by a decrease in β -pinene and limonene; α -pinene and myrcene change, but not as markedly.

II. Sierra-Pacific Region

Northward into the Sierra Nevada and the Coast Range, the transition zone quickly merges with the second region—Sierra-Pacific. Plot composition is characterized by more evenly balanced amounts of 3-carene, β -pinene, myrcene, and limonene; the latter two are somewhat lower than the first two, but α -pinene remains low. This composition remains quite constant for several hundred miles northward to the next transition zone.

The coastal plots of the region, 31–35, differ slightly but consistently from the Sierra Nevada plots, 26, 27, 36, 37, 39. On the coast, β -pinene is greater than 3-carene, whereas in the Sierra Nevada, 3-carene is greater than β -pinene. With the greater amount of β -pinene, the coastal portion of the region is more similar to Region I to the south; with the greater amount of 3-carene, the Sierra portion is more similar to Region III to the north. The line of shift between the two portions of the region appears to run northward along and beyond the Sacramento River. Plot 28, on the eastern slopes of the Sierra Nevada and separated from the main block of the tree's range by the Sierra Crest, does not fit the Sierra-Pacific Region, differing from the nearest plot by three components. It has more of the characteristics of the next transition zone, Modoc-Siskiyou.

B. Modoc-Siskiyou Transition Zone

The Modoc-Siskiyou transition zone is not so clearly defined as the Tehachapi zone. Plots within it—25, 28, 38, 40—show increased amounts of 3-carene with decreases in β -pinene and limonene. Myrcene and α -pinene also decrease, but not as greatly. The absence of plots to the north and west injects some uncertainty into the placement of those boundaries of the zone. From other data (Smith and others, 1969), however, it is apparent that plots in Oregon north of the Siskiyou resemble those of the coastal portion of the Sierra-Pacific Region.

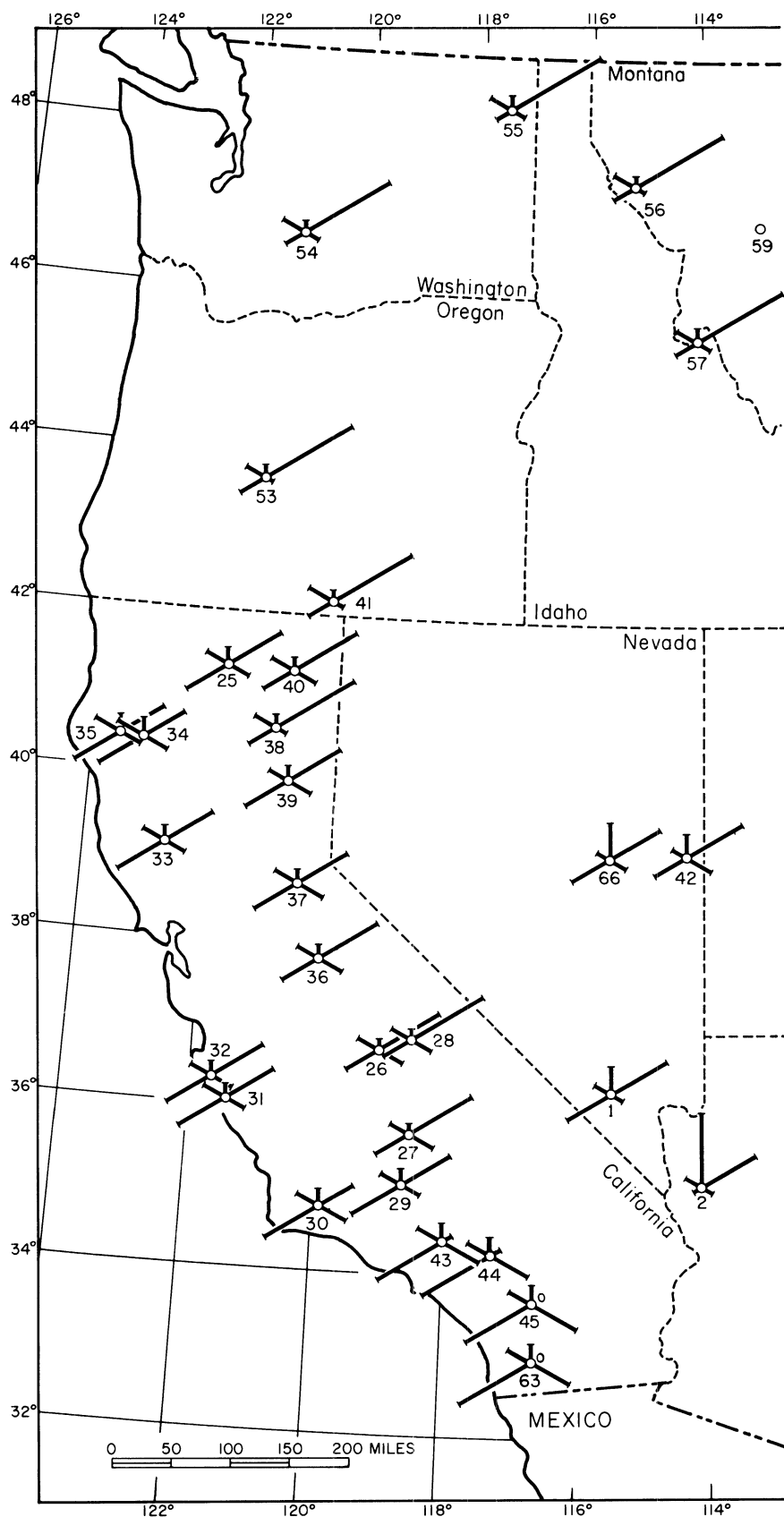
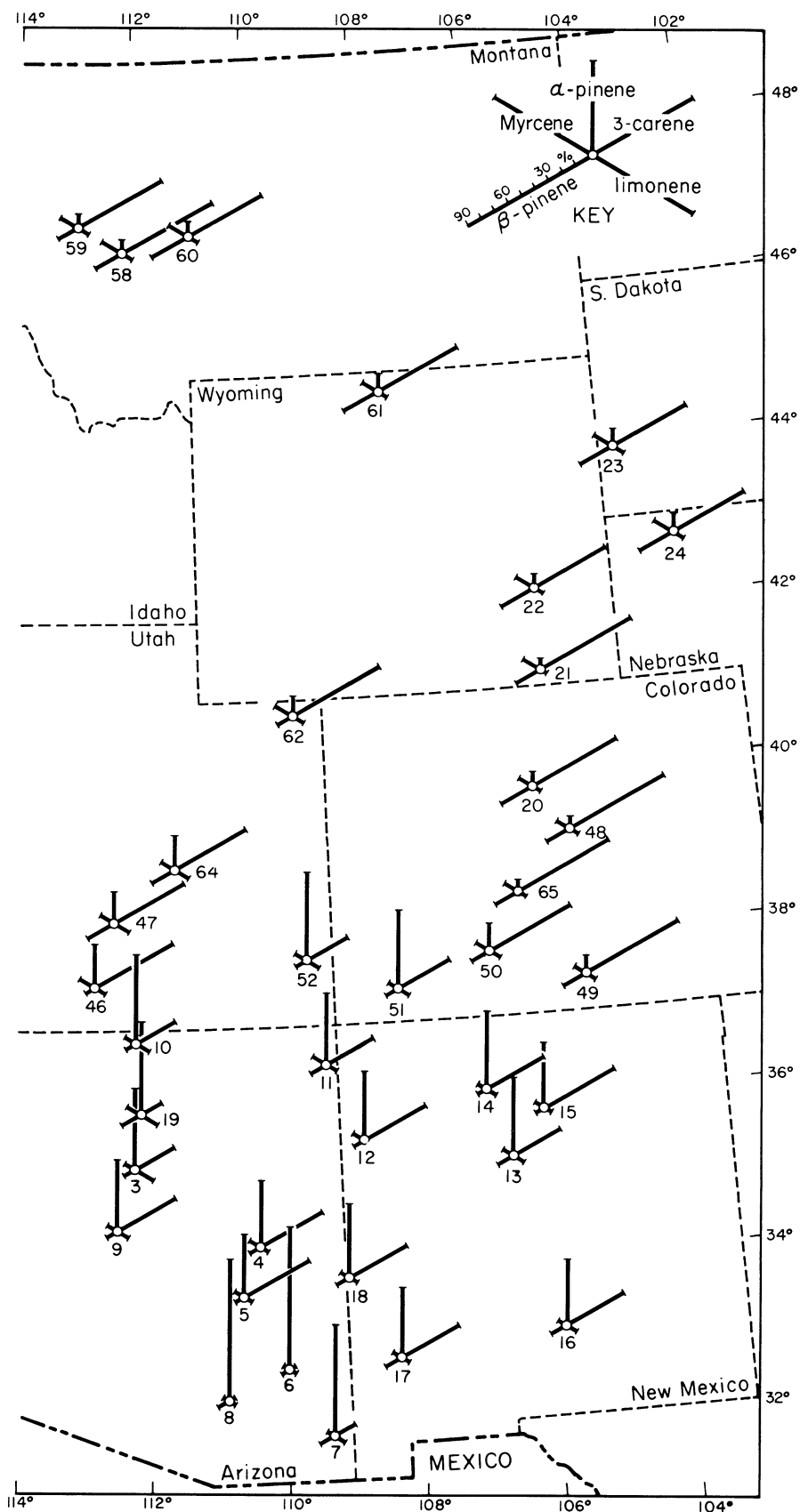


Figure 5.—Average amounts of the major monoterpene components— α -pinene, β -pinene, 3-carene, myrcene, limonene—



at 66 sites in Western United States are shown by length of bars. Site number is shown by each bar cluster.

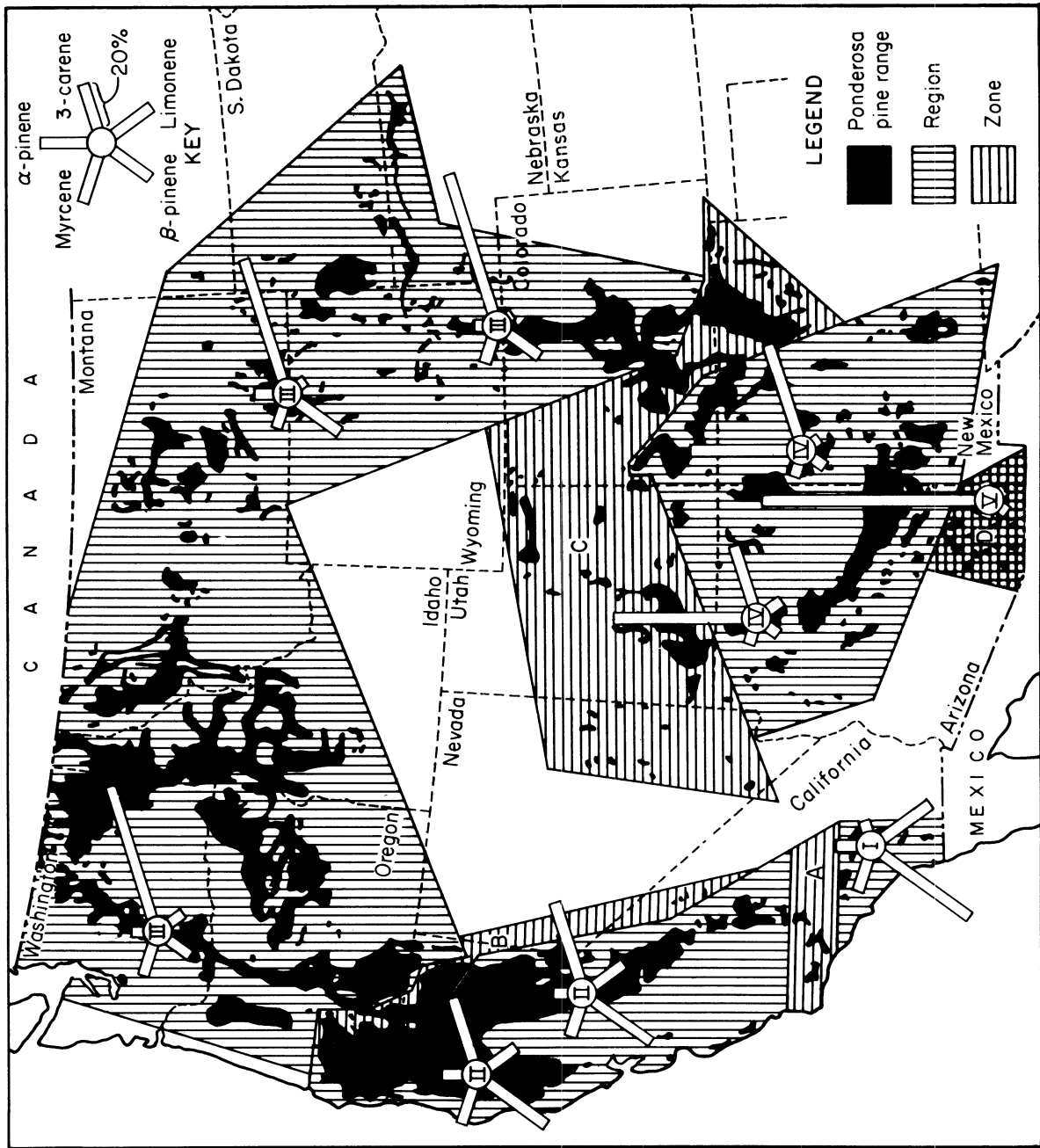


Figure 6.—The monoterpane composition of typical plots in the five regions: I, San Jacinto; II, Sierra-Pacific; III, Cascade-Northern; IV, Mogollon; V, Coronado. The four transition zones are: A, Tehachapi; B, Modoc-Siskiyou; C, Escalante-Rio Grande; D, Chiricahua.

Regions	Regions																			
	II					III					IV					V				
	α	β	3	M	L	α	β	3	M	L	α	β	3	M	L	α	β	3	M	L
I San Jacinto	x	x	x	-	x	x	x	-	x	x	x	x	x	x	x	x	x	-	x	x
II Sierra-Pacific						x	x	x	-	x	x	x	x	x	x	x	x	x	x	x
III Cascade-Northern											x	x	x	x	x	x	x	x	x	x
IV Mogollon																	x	-	x	x
V Coronado																				
x significantly different at 95% level																				
- below 95% level																				
α = α -pinene, β = β -pinene, 3 = 3-carene																				
M = myrcene, L = limonene																				

Figure 7.—Differences at the 95 percent level of confidence between typical plots in each region for each of the five major monoterpene components.

III. Cascade-Northern Region

Northward and eastward from the Modoc-Siskiyou transition zone, the composition type quickly becomes that of the Cascade-Northern Region. Plots are characterized by a large amount of 3-carene, moderate amounts of myrcene and β -pinene, and small amounts of α -pinene and limonene. This composition, with the exception of two minor shifts, remains unchanged over a large portion of the range of the species, across the northern tier of States, and southward to southern Colorado. The first minor shift is at the Missouri River between plots 58 and 60, where β -pinene increases and limonene decreases. The second is between plot 22 in the Laramie Range and plot 21 in the Medicine Bow Mountains, where the plot characteristics again closely resemble those of plots west of the Missouri River, except for a continued decrease in limonene.

C. Escalante-Rio Grande Transition Zone

The third transition zone, Escalante-Rio Grande, is by far the largest. It extends from northeastern New Mexico, northwest across Colorado, then west through Utah into eastern and southern Nevada. Here, compared to Region III, there is a rapid decrease in 3-carene and a balancing increase in α -pinene; β -pinene, myrcene, and limonene remain low. There is still the interplot variability which characterizes transition zones, however. More plots are needed to position the southern boundary of the zone.

This extensive zone could be the subject of additional research. The western portion of it appears to

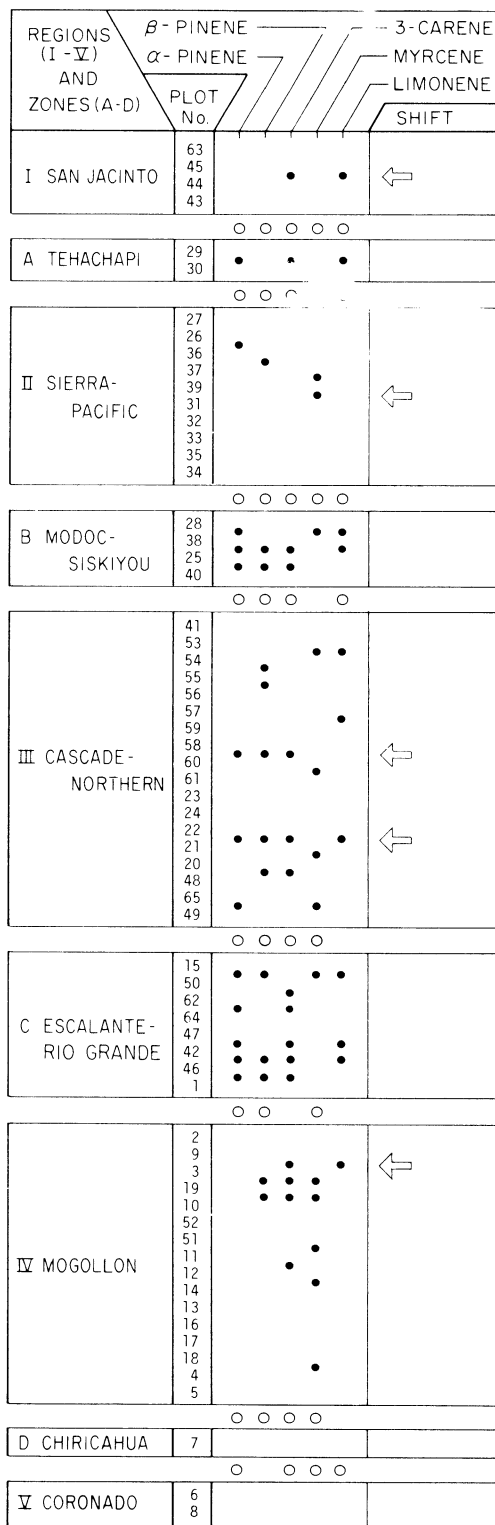


Figure 8.—Amounts of monoterpenes in ponderosa pines differed between adjoining plots in each region and zone. The solid circle designates a significant difference (95 percent confidence level) between plots within regions and zones for the monoterpenes shown; the open circle denotes significance between regions and zones; arrow locates shifts within regions.

be a "melting pot" of ponderosa pine. Plots in it bear some resemblance to virtually every region: α -pinene in plots 46, 47, 64 from Regions IV and V; 3-carene in plot 50 from Region III; β -pinene and limonene in plots 1 and 42 from Regions I and II. One might attribute this great variability to the fragmented occurrence of the tree; but other parts of the tree's range are as heavily fragmented without such variability. Some of the stands in this zone have been isolated for so long from larger blocks of ponderosa that new chemical varieties may be evolving. Man has inadvertently contributed by removing large portions of these blocks for mining.

IV. Mogollon Region

Region IV, Mogollon, is southward of the Escalante-Rio Grande transition zone. This region is characterized by large amounts of α -pinene and 3-carene and small amounts of β -pinene, myrcene, and limonene. Except for the northwest portion of this region, plots show nearly equal amounts of α -pinene and 3-carene. In the northwest portion, however, there is a shift to plots—3, 19, 10, 52—with an increase in α -pinene and limonene and a decrease in 3-carene. This is the only intraregional shift in which two components change so greatly at the same time, and it might be argued that the changes are large enough to establish another region or subregion. As these plots bear strong resemblance to the rest of the region, however, it seems best not to subdivide. Of the four plots, only plot 19 is appreciably different from the others. Further work is needed to define this part of the region. The eastern and southern parts of this region fit the requirements of a region quite well.

V. Coronado Region and D. Chiricahua Zone

Region V, Coronado, is south of Region IV. There is no intervening transition zone, at least as defined in this paper, though the Gila River serves as a boundary between the two regions. Of the three plots in the area, 6 and 8 are considered typical of the region; monoterpene composition in these plots is about 90 percent α -pinene with small or trace amounts of the other four major components. There are no differences between the two plots.

Plot 7 resembles both Coronado and Mogollon, but differs significantly from both; therefore the single plot has been designated a transition zone and named Chiricahua. Peloquin (1971) studied the area more extensively and found the Mogollon type

of composition at the upper elevational limit of ponderosa pine; plots 6, 7, 8 are at a lower elevation. Thus in this region there is an elevational trend with the Coronado type of composition at lower elevations, the Mogollon type at higher elevations, and a transitional type between. Peloquin suggests that transition plots indicate a mixture or hybridization, or both, of the two types; his terms, Arizona and southwestern variety of ponderosa pine, fit very closely to the Coronado and Mogollon types, respectively, of this classification. Peloquin also found one stand of the Mogollon type at a lower elevation than the Coronado type.

This classification is somewhat similar to an earlier one (Smith and others 1969); it differs most notably in establishing transition zones and in defining more clearly the regional limits.

The regions only partially correspond to any of the existing subdivisions based on growth and morphological characteristics of ponderosa pine. Wells (1964) shows no division in California except to include the extreme northeastern corner in the Northern Plateau division; he separates the Cascade-Northern type into two ecotypes, shows no transition zones, and delineates the Mogollon type differently. The sampling of Squillace and Silen (1962) was inadequate to detect some of the type differences, although this system does separate Sierra-Pacific from Cascade-Northern.

The proposed classification agrees surprisingly well with the six climatic regions established by Weidman (1939): Regions I and II resemble Weidman's region 6; region III coincides well with his regions 1, 2, and 5; regions IV and V bear some resemblance to his region 4; zone C partially fits his region 3. Neither the traditional geographic classification nor any of the ecotype classifications specify transition zones, although transitions might be implied in ecotype studies.

The taxonomy of ponderosa pine has never become fixed for any prolonged period. The most commonly cited classification is that of var. *ponderosa* on the Pacific Coast, var. *scopulorum* in the Rocky Mountains, and var. *arizonica* in southeastern Arizona. At times var. *arizonica* has been given full species status (Mirov 1961). The regional and transitional subdivision of the present report differs considerably from this classification.

Genetic Hypothesis

The use of average monoterpene composition to delineate regions and transition zones is one way to

summarize concisely the complex data on variation. Averages, however, conceal a tremendous richness in intraspecific variation that characterizes ponderosa pine. To display this richness it is necessary to focus on individual tree types of monoterpene composition. However, some basis for such a classification must be established.

It is strongly emphasized that the hypothesis which is proposed in this section is based on somewhat fragmentary data. It is formulated from circumstantial evidence in this report and from scattered, though apparently relevant, data from the literature. Supporting data from controlled breeding experiments have not yet been developed. Nevertheless, there does seem to be some grounds for the hypothesis.

Several investigators have proposed a complex mechanism for one or more terpenes in a coniferous resin system: for Scotch pine, *Pinus sylvestris* L. (Tobolski and Hanover 1971); for slash pine (Squillace and Fisher 1966); for white spruce, *Picea glauca* Moench (Wilkinson and others 1971). Others have proposed a simple mechanism. Hanover (1966) proposed dominant and recessive alleles for 3-carene in the cortical tissue of western white pine. Squillace (1971) suggested much the same mechanism for β -pinene and myrcene in the branch cortex of slash pine. Forde (1964) proposed one incompletely dominant gene governing the major terpene difference between Monterey pine and knobcone pine (*Pinus attenuata* Lemm.), but included a multifactorial system of modifiers to account for deviations from expected values.

Two results of the present study—the frequency distribution of the percentage of the five major monoterpenes and the percent terpene in whole resin—suggest, in the absence of controlled breeding tests, a genetic mechanism controlling monoterpene composition. Though basically simple, the hypothesis seems to account for much of the large variation found.

First, from figure 3, three fairly well-defined levels of frequency can be observed for each of the five major monoterpenes (table 9). As proposed here, the three levels are caused by an additive pair of alleles, one with a value of 0 and the other with a value of 1 for the synthesis of a particular component. Combinations of these two alleles—00, 01, and 11—produce the three levels observed. The three levels are not equally discernible in all five components. They are most sharply defined in α -pinene and least evident in myrcene and limonene. Apparently, a small amount of each terpene is produced at

the lowest level; that is, at the 00 combination. There are 243 theoretical combinations of three levels of five components, but only 169 can occur. The other combinations cannot occur because the minimum values taken from table 9 exceed 100 percent, or the maximum values fail to reach 100 percent with allowances of 3 percent for minor components. For example, when all components are at the lowest level, there is a maximum of only 50 percent, and when all are at the highest level, the minimum is 166 percent. In this study, 115 combinations, or 69 percent of those possible, were found.

Table 9.—Intervals of normalized monoterpene composition used to determine the code values of each component for defining ponderosa pine composition types

Monoterpene component	Code value		
	0	1	2
	Interval		
	Percent		
α -pinene	0 to 17.4	17.5 to 64.4	64.5 to 100
β -pinene	0 to 4.4	4.5 to 35.4	35.5 to 100
3-carene	0 to 15.4	15.5 to 35.4	35.5 to 100
Myrcene	0 to 2.4	2.5 to 15.4	15.5 to 100
Limonene	0 to 2.4	2.5 to 17.4	17.5 to 100

Secondly, the relatively constant percentage of the terpene fraction of whole resin, despite wide fluctuations in the individual monoterpenes, suggests the production of a constant amount of the monoterpene precursor; this is available to the particular array of alleles of the five major monoterpenes. One would also have to speculate that the five major monoterpene genes do not compete equally for the precursor. The data on the frequency levels (table 9) suggest that α -pinene is about twice as competitive as β -pinene and 3-carene and about four times as competitive as myrcene and limonene. Therefore, the percentage of each component in a given tree is dependent on both its pair of alleles and on the pairs of alleles for the other four components. This competition among the five components for a relatively fixed amount of precursor within the framework of the genetic mechanism proposed could be the “multifactorial system of modifiers” suggested by Forde (1964).

The variation in competitiveness and the 115 combinations could account for the broad frequency distributions found in the study. Irregularities in the biosynthetic processes, as well as an accumulation of small effects caused by a number of physical and biological factors, might spread this distribution even more. The other monoterpenes found in ponderosa pine, and discussed as minor components later in this report, occur in small amounts, usually less than 3 percent; these could be produced either by "spin-offs" from the synthesis of the major components or by a pair of alleles that is always 00.

Alternatives to this hypothesis were considered. A second and somewhat similar hypothesis would be a dominant and recessive pair of alleles, giving 32 theoretical combinations. Other hypotheses would include combinations of the two just described. When the data were analyzed, a greater percent of the possible types of composition were found with the dominant-recessive allele combinations than with the additive alleles (table 10). On the other hand greater differences also were found between sets of trees of the same compositional type from different localities with the dominant-recessive combinations than with the additives. The lesser variation within sets of trees of the same compositional type would appear to be additional support for selection of the additive allele hypothesis.

One difficulty in this classification is the absence of certain expected combinations in some plots. This is particularly true in plots having many samples in the 01 heterozygous grouping; here there should be some homozygous 00's and 11's. Their absence could be explained either by inadequate sampling or by inaccuracy in the placement of the dividing point between groups; that is, an inflexible criterion may have been applied to a natural phenomenon that has some flexibility.

This concept of xylem terpene genetic control might be applied to other pines. It could account for the near loss of a monoterpene component when a hybrid with a measurable amount of it is backcrossed to the parent species lacking it. This particular sequence could explain the loss of heptane in a series of backcrosses of hybrids of Jeffrey pine (*Pinus jeffreyi* Grev. and Balf.), ponderosa pine, and Coulter pine (*P. coulteri* D. Don); the failure to reach the expected intermediate amount in certain crosses; and the difference found between full siblings (Smith 1967).

Individual Tree Types

The genetic hypothesis provides a basis for the classification of individual trees. The 0, 1, 2 codes values in table 9 were applied to the five major monoterpenes so that each tree was assigned a five-digit number reflecting the percentage level of each monoterpene. Thus a tree with a composition of 5 percent α -pinene, 20 percent β -pinene, 60 percent 3-carene, 2 percent myrcene, and 13 percent limonene would be given the number 01201 for those five components:

0 = α -pinene, 0 to 17.4 percent

1 = β -pinene, 4.5 to 35.4 percent

2 = 3-carene, 35.5 + percent

0 = myrcene, 0 to 2.4 percent

1 = limonene, 2.5 to 17.4 percent

As noted earlier, 115 types of combinations were found in the 4,894 trees in the full plots in the study.

Table 10—Alternative hypothesis of the genetic basis of monoterpene composition in ponderosa pine: combinations of additive (Add) and dominant/recessive (D/r) pairs of alleles, by theoretical, possible, and actual numbers of tree types found in 4,894 trees

α -pinene	β -pinene	3-carene	Myrcene	Limonene	<u>Tree types</u>			Ratio of actual to possible types
<u>Genetic mechanism</u>					Theoretical	Possible	Actually found	
D/r	D/r	D/r	D/r	D/r	<u>Number</u>			Percent
Add	D/r	D/r	D/r	D/r	32	31	26	84
Add	Add	Add	D/r	D/r	48	47	39	83
Add	Add	Add	Add	D/r	108	83	69	83
Add	Add	Add	Add	Add	243	169	115	68

However, the distribution of these types is quite unequal. Thirty percent of the trees consist of two common types—01210 and 01211. Sixty-four percent consist of these two and 12 other types, each represented by at least 100 trees. Ninety-five percent of the trees consist of only 54 types.

Thirty individual tree types were found 43 times or more in the 4,894-tree sample of ponderosa pine (fig. 9A); 29 types, 9 to 36 times (fig. 9B); and 32 types, 3 to 8 times (fig. 9C). Twenty-four additional types were found only once or twice and must be classified as rare (table 11). If a typical ponderosa pine had to be selected, it would be the 01211, with an average percent composition of 6–21–51–10–9 respectively for a α -pinene, β -pinene, 3-carene, myrcene, and limonene. This type is found in four of the five regions and three of the four zones. The modal tree of each region is found in three to six other regions and zones (table 12).

Many types were found in two or three regions and the intervening zones (fig. 9A, B, C). Most of

the types found more than 25 times—and therefore in numbers sufficient for making valid observations—have a very noticeable clinal distribution, with two basic inclinations: a decrease in the frequency of a type outward from a region or zone to the regions or zones on either side, and a decrease in one direction from a region or zone (fig. 9A, B, C). Thus, the species is largely composed of a number of individual monoterpene types whose abundance is clined to varying degrees throughout the species' distribution. Thirty-two types that occur more than about 25 times, and constitute 83 percent of the total sample of trees, show this characteristic. Less than 25 trees seems insufficient to show clines. The number of different tree types and their distribution would appear to be the cause of the geographic variation in ponderosa pine xylem monoterpenes. The large number of types of composition at a location would create an unstable condition since changes in the frequency of types would be quite possible through the action of man or other pro-

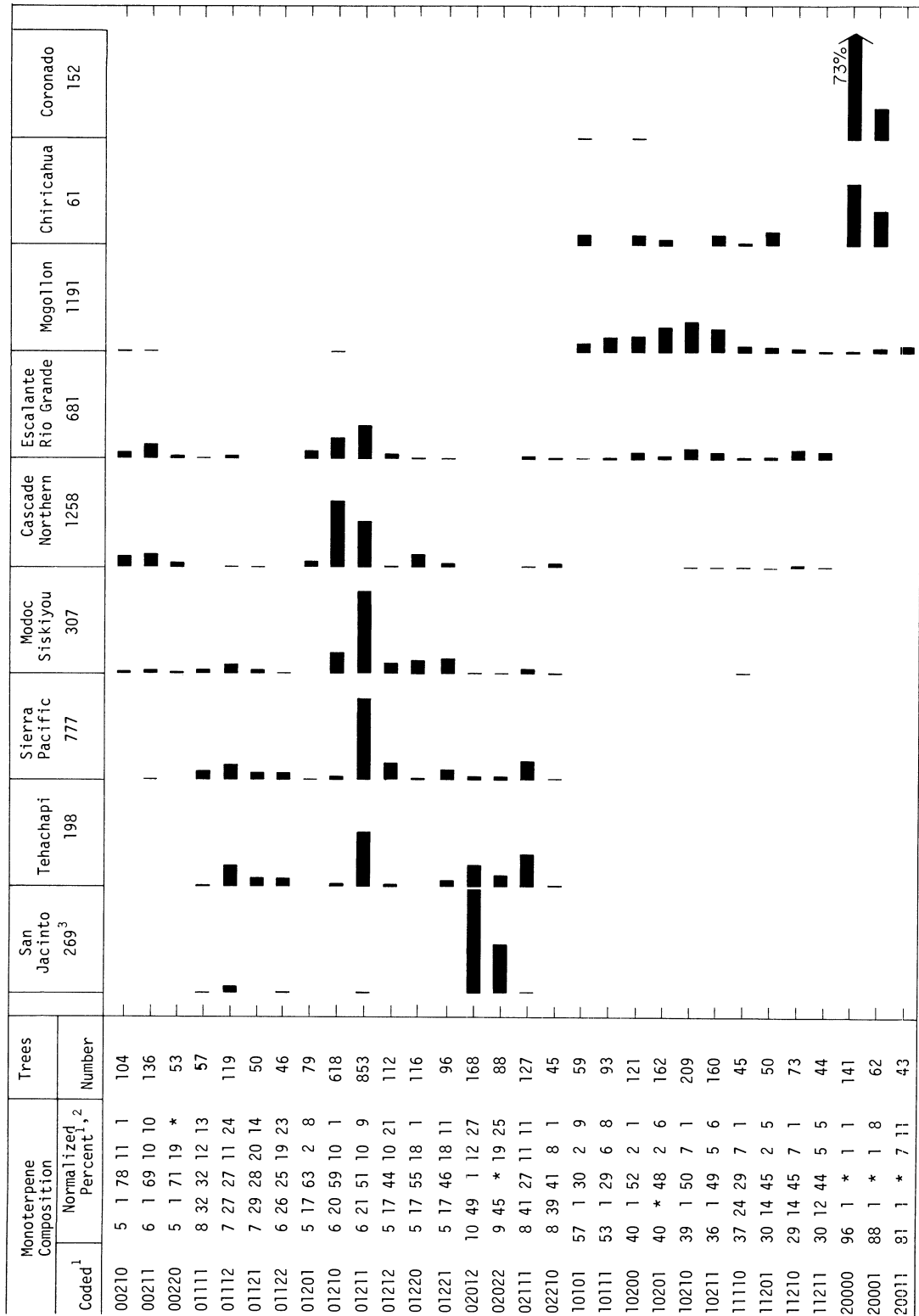
Table 11.—The coded type and normalized monoterpene composition of the 24 ponderosa pine types found only once or twice in a 4,894-tree sample

Code type ¹	No. of trees	Plot No.	Normalized composition ²				
			α -pinene	β -pinene	3-carene	Myrcene	Limonene
			Percent ²				
00022	1	68	11	3	0	16	70
00112	1	42	8	0	32	8	49
00200	2	50,61	6	4	84	2	0
00202	2	16,47	7	0	64	2	23
01102	2	47,42	13	29	32	2	21
01110	1	1	17	32	33	13	1
02100	1	30	11	50	34	2	1
02122	2	35,37	7	37	18	18	19
02220	1	33	8	37	36	17	0
10001 ³	1	7	59	1	0	2	10
10020	1	34	42	4	0	51	1
10021	2	17,17	64	1	2	16	17
11001	2	7,7	61	27	0	1	10
11002	2	19,51	53	23	0	1	21
11012	1	19	45	21	0	7	26
11102	1	42	24	21	21	2	30
11120	1	24	44	13	23	17	1
11202	2	11,47	23	11	42	2	19
11212	1	42	19	8	45	5	18
12022	1	30	19	38	0	20	21
12210	1	60	20	37	37	6	0
21010	1	19	73	19	0	7	1
21011	1	52	75	7	0	4	13
21100	1	16	71	11	16	1	0

¹ Digits represent, left to right, α -pinene, β -pinene, 3-carene, myrcene, limonene. See table 9 for explanation.

² Includes small amounts of minor components not listed.

³ Also contains 15 percent sabinene and 13 percent terpinolene.



¹Respectively from left to right: α -pinene, β -pinene, 3-carene, myrcene, limonene.

²Includes small amounts of minor components not listed; * = less than 0.5.

³Total number of trees in Region or Zone for all three parts of this figure and Table 10.

Figure 9.—Geographic distribution for 4,894 ponderosa pines classified on the basis of monoterpene composition (see table 9). Distributions are normalized within each region and zone in its entirety.—Continued on p. 29.

A. Types occurring 43 times or more.

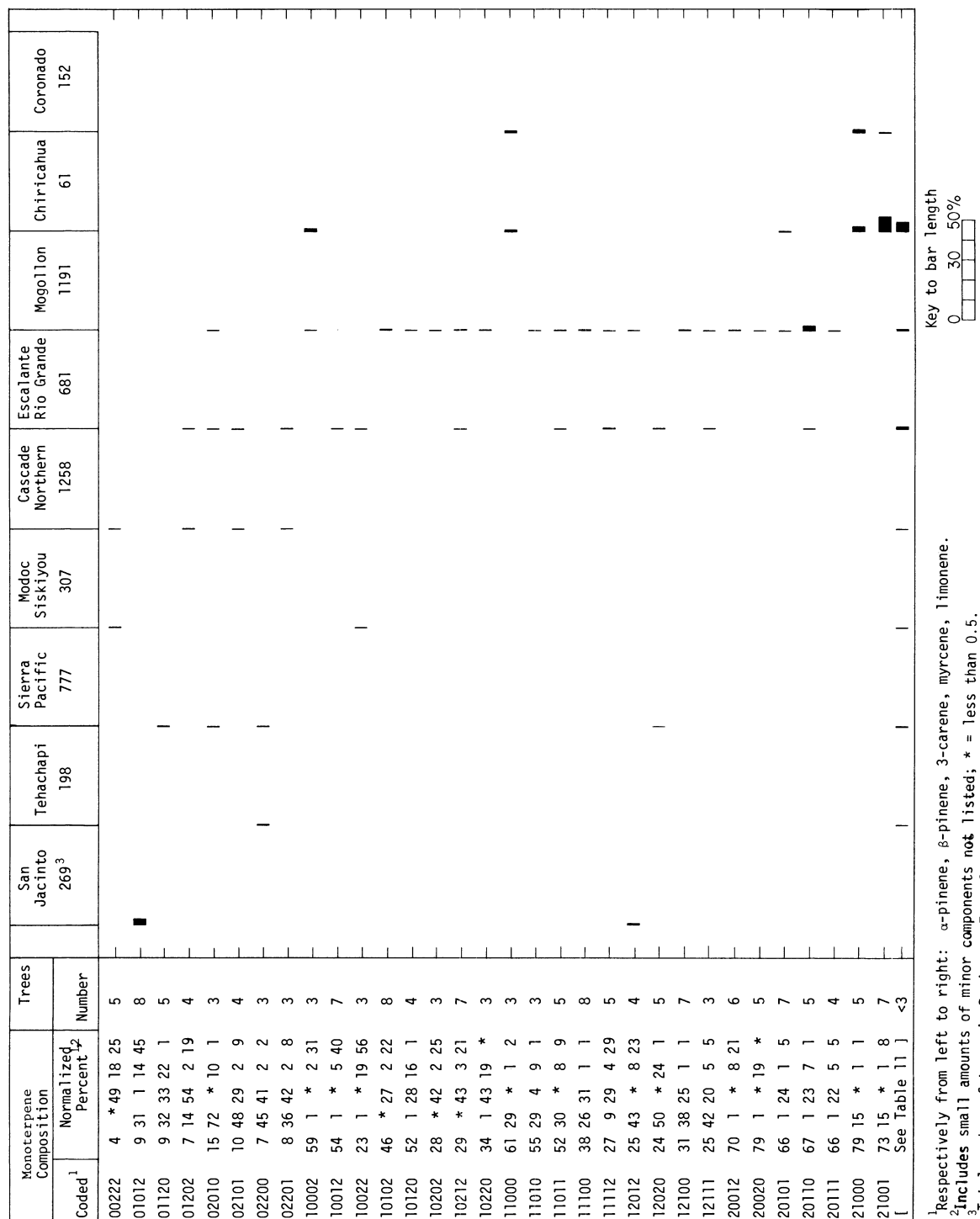


Figure 9.—Continued from p. 28. C. Occurring 3 to 8 times.

cesses, resulting in a different kind of current and subsequent stand with respect to the terpene composition. This possibility for change is particularly true of transition zones.

The distribution of individual tree types shows, as do the plot averages reported earlier, that zones are much more variable than regions. Regions I, San Jacinto, and V, Coronado, have the fewest tree types—15 and 10 respectively; in contrast, 20 to 30 types occur in individual plots in the Escalante-Rio Grande zone, C. The average numbers of tree types per plot for regions I to V are 9, 17, 12, 23, 7; and for zones A to D are 18, 16, 22, 17. This suggests

most stability in regions I, III, and V, partial instability in II and IV, and high instability of the transition zones.

The “melting pot” characteristic of the Escalante-Rio Grande zone is strongly evident in the number of monoterpane types found there. Of the 91 most common types (fig. 9), 59 are found in this zone; and it contains examples of one or more types of composition found in each of the other regions and zones. Thus, this zone is literally tied to all regions and zones.

Sets of trees of the same composition type appearing in different plots, regions, or zones differed less

Table 12.—*The code type and actual monoterpene composition of the modal tree in each region; its occurrence in that region (underlined) and in other regions and zones*

Region	Code type ¹	Composition ¹										
		Actual		Occurrence in regions and zones								
				I	A	II	B	III	C	IV	D	V
		Percent		Percent ²								
I	02012	10	49	1	12	27						
II	01211	6	21	51	10	9						
III	01210	6	20	59	10	1						
IV	10210	39	1	50	7	1						
V	20000	96	1	1	0	1	1				25	64

¹ From left to right: α -pinene, β -pinene, 3-carene, myrcene, limonene.

² No entry = 0; values are from normalization of all types of trees for each region and zone from figure 9 and table 11.

Table 13.—*Occurrence of coded composition types in three pairs of plots with near-identical average composition: See figure 9 for actual composition of coded types*

<i>Plots 6 and 8</i>			<i>Plots 43 and 44</i>			<i>Plots 56 and 59</i>					
<i>Coded</i>	<i>Plot</i>		<i>Coded</i>	<i>Plot</i>		<i>Coded</i>	<i>Plot</i>				
<i>types</i>	6	8	<i>types</i>	43	44	<i>types</i>	56	59			
	<i>Percent</i>			<i>Percent</i>			<i>Percent</i>				
10101 ¹	0	1	01012	1	4	00210	9	10			
10200	1	0	01022	8	0	00211	8	14			
11000	0	3	01111	0	1	00212	0	1			
12000	3	8	01112	4	7	00220	6	6			
20000	79	67	01122	1	1	00221	1	3			
20001	12	18	01211	0	1	01222	1	0			
20100	1	0	02011	9	3	01200	0	1			
20101	0	1	02012	36	50	01210	41	40			
21000	3	1	02020	0	1	01211	21	19			
21001	1	0	02021	8	11	01212	1	0			
			02022	33	16	01220	4	3			
			02111	1	0	11221	8	0			
			02112	0	4	11210	0	1			
Plot: Mean composition ¹			Plot: Mean composition ¹			Plot: Mean composition ¹					
6:	92	3	1	1	2	56:	4	13	62	12	4
8:	90	5	1	1	1	59:	5	13	63	11	4

¹ From left to right: α -pinene, β -pinene, 3-carene, myrcene, limonene; includes small amounts of minor components not listed.

Table 14.—Composition of three tree types showing high levels of limonene, by tree and plot number

Tree No.	Plot No.	α -pinene	β -pinene	3-carene	Myrcene	Limonene
Percent ¹						
High in limonene						
3139	40	18	1	0	22	59
3298	42	26	— ²	0	16	58
3352	42	24	— ²	0	18	57
5361	68	12	3	0	15	70
A-78	40A	22	3	0	18	57
High in limonene and medium in β -pinene						
3547	45	9	19	0	15	56
A-95	40A	21	23	0	12	43
A-315	40A	15	19	0	21	45
Medium high in limonene and α -pinene						
82	2	62	1	0	3	33
129	2	61	— ²	0	3	36
172	3	63	— ²	0	3	33
175	3	60	1	0	2	37
196	3	55	— ²	0	3	42
711	9	67	1	0	1	30
1497	19	53	1	0	3	43

¹Includes small amounts of minor components not listed.²Trace.

than 2 percent of the time in any major component, despite the possible wide variation which could occur within the structure of the coding system. This adds very strong support to the coding classification system and to the genetic hypothesis.

Sets of trees of different composition types appearing in the same plot, region, or zone always show a significant difference in the means of one or more components.

The typical tree (the mode) and the average tree (the mean) in a region may not be the same. The type trees in regions II and III differ only in the level of limonene, yet the average trees differ significantly in α -pinene, β -pinene and 3-carene as well as limonene. The type trees in II and IV differ in α -pinene, β -pinene, and limonene, yet the average trees differ in all five major monoterpene components. The kinds and numbers of other tree types account for this. The typical tree of a region, zone, or plot, can be significantly different, in the level of four or five major monoterpenes, from other tree types in the same region, plot, or zone.

Plots that do not differ essentially in average monoterpene composition always show some differences in the tree types they contain and in the frequency of types (table 13). Thus, the 80-tree sample is adequate for obtaining regional and local averages

but not for finding the numbers and frequencies of tree types.

Rare types of composition occur most commonly in C and IV, with eight each; less commonly in A, II, III, and D, with two to three each; and not at all in I, B, V (table 11).

With such a wide assortment of tree types, it is easy to see why plots vary so much—even with 80-tree samples. Any study of xylem resin terpenes must take into account individual tree data as well as plot averages.

The large number and variation of composition types in zones explains, at least partially, the results of the plot size study in the section on procedures. Plot 40 which was used in that study is in a transition zone. A similar study in a region would probably show even less difference between plots.

High Limonene Trees

Trees of high limonene composition are of particular interest because of their possible resistance to western pine beetle. Three studies had three results: (1) Of the five major terpenes of ponderosa pine, limonene was the most toxic to adult beetles (Smith 1965); (2) trees with the highest percentage of limonene were the least frequently attacked and

killed by the beetle (Smith 1966); (3) in forced attack studies, beetles were far less successful on trees with a higher percentage of limonene—the limonene content of the monoterpenes of these trees was about 25 percent (Smith 1969).

Five trees with much higher levels of limonene were found in this survey. All had about the same terpene composition: Approximately 20 percent α -pinene, 20 percent myrcene, 60 percent limonene and small or trace amounts of β -pinene and 3-carene (table 14). Samples taken at different heights on three of these trees showed the composition to be constant within each tree. Two of the trees are 18 to 24 inches d.b.h. and the other three are 8 to 12 inches.

These special trees (from plots 40, 40A, 42, and 68) were found in different geographical areas and the trees with which they are associated are different. Plot 40 is a mixture of ponderosa with Jeffrey pine and is in a fairly continuous block of pine which extends for hundreds of miles. Plot 42 is an isolated small group of ponderosa with no hard pine associates. Plot 68 is a mixture of ponderosa, Coulter pine (*P. coulteri*), and Digger (*P. sabiniana* Dougl.), and ponderosa pine is considerably more extensive there than in plot 42 but much less so than in plot 40. The three plots differ from each other in the frequency distribution of the five major terpenes (fig. 3).

All three areas where these high-limonene trees occur have three characteristics in common: Each shows a moderately high level of limonene, including individual trees with relatively high limonene; each has trees lacking either β -pinene or 3-carene; and each is located in a transition zone.

The hypothesis is here formulated that with a two-allele mechanism controlling each terpene, trees lacking either β -pinene or 3-carene can produce offspring lacking both compounds; about one-fourth of the progeny would lack both β -pinene and 3-carene in such a cross. When this happens α -pinene, myrcene, and limonene dominate monoterpene synthesis; and each can occur as a large percentage of the monoterpene composition, depending on the makeup of its pair of alleles and the pairs of the other two components.

All three types of trees lacking both 3-carene and β -pinene have been found—high limonene, high myrcene, and high α -pinene. The high-limonene trees have been noted. One tree with 51 percent myrcene was found in plot 34 (type 10020 in table 11). This plot meets the requirement of including both kinds of proposed parents—trees lacking β -

pinene or 3-carene—but it is not in a transition zone. Several trees having high limonene and α -pinene—with an approximate composition of 60 percent α -pinene, 0 percent β -pinene, 0 percent 3-carene, 0 percent myrcene, and 40 percent limonene—have been found in plots 2, 3, 9, 19 (table 14). Here, too, these plots meet the requirements but ~~are not~~ in a transition zone. An additional piece of supporting evidence appears in plot 42; two high-limonene trees were found along with the highest proportional number of the required parent trees in the 80-tree sample; the level of limonene in the plot is moderately high.

The proposed mechanism is hypothetical and no conclusive evidence has been obtained from controlled breeding to support it. Also, few plots with a generally high level of limonene include trees lacking β -pinene and trees lacking 3-carene. Therefore the natural occurrence of high-limonene trees would have to be presumed rare.

An additional 624 trees were sampled around plot 40 to see if other high-limonene trees could be found; and one more such tree was found, along with several trees which seemed to be about midway between a high-limonene tree and one which might be called above-average for a plot (table 14). Thus, in this plot area, one high-limonene tree was found in the primary 80-tree sample, but only one more was found in an additional sample eight times as large. The 624-tree sample and the original 80-tree sample had about the same proportional number of trees lacking either β -pinene or 3-carene. The second high-limonene tree found in the vicinity of plot 40 and several small trees with a medium-high limonene level are in a dense pole-size stand near the first high-limonene tree. The first high-limonene tree may be one of the parents of some of the pole-sized stand.

Minor Monoterpene Components

Six monoterpenes and a related hydrocarbon are classed as minor components: α -thujene, camphene, sabinene, β -phellandrene, γ -terpinene, terpinolene, and the related hydrocarbon, heptane. The relegation of components to a minor category is somewhat arbitrary. For example, in individual trees and plots one or more major component could be classed as minor. In this report minor components are those which are never more than 5 percent in a tree and never more than 3 percent in a plot. In certain exceptional trees, however, four minor components occur in larger amounts: sabinene and terpinolene in

Table 15.—*Monoterpene composition of ponderosa pine characterized by unusual amounts of minor components, by tree number*

Tree No.	α -pinene	Camphene	β -pinene	3-carene	Sabinene	Myrcene	Limonene	β -phellandrene	γ -terpinene	Terpinolene
Percent										
High in sabinene and terpinolene ¹										
486	78	1	²	²	3	1	12	²	0	5
531	59	²	19	0	8	1	2	1	0	10
543	59	²	²	0	15	2	10	²	1	13
High in β -phellandrene										
3486	8	0	37	0	0	21	18	16	0	0
3501	15	0	45	0	0	9	21	10	0	0
3502	12	0	49	0	0	7	18	13	0	0
High in camphene										
2044	5	10	24	32	²	9	17	1	²	2
2128	5	12	26	30	²	15	8	1	²	2

¹ These three trees were selected to represent 11 of this type.² Trace.

11 trees in plot 7, zone D; β -phellandrene in three trees in plot 41, region I; and camphene in one tree each in plots 26 and 27, region II (table 15). Usually, together the minor components comprise about 3 percent of the total monoterpenes in most trees.

The quantification and qualification of these components are not precise. Small peaks on the chromatogram are difficult to measure with the accuracy of larger peaks; their small size also increases the difficulty of single-peak preparation. These minor peaks are often partially obscured by the trailing or leading edge of the peak of a major component, thus adding to the difficulty.

Sabinene is a minor component in all regions; it is often difficult to separate from 3-carene because it elutes on the trailing edge of the 3-carene peak. It is almost always found in trace amounts except in region V and zone D, where it is frequently between 1 and 2 percent. In 11 trees in plot 7, Chiricahua zone, it ranged from 3 to 15 percent (table 15). In such trees there is a nearly 1:1 ratio between sabinene and terpinolene, and therefore terpinolene also becomes an exception to the rule in these same trees. Because sabinene is found in measurable amounts only in region V and zone D, and then only in trees not typical, its presence may indicate either an intraspecific hybrid or an interspecific hybrid

with some pine having a considerable amount of sabinene. One pine in this area which might hybridize with ponderosa is Apache pine (*P. engelmannii* Carr.), but this pine has not been analyzed sufficiently to determine whether it contains sabinene.

Terpinolene is the most common and abundant minor component. As it is directly associated with 3-carene, it is most abundant in those trees and plots with a large amount of 3-carene and is absent from trees lacking 3-carene. In most trees, it ranges from 1 to 3 percent except in the same 11 trees in plot 7 noted under sabinene, where it ranges from 3 to 15 percent and has a nearly 1:1 ratio with sabinene. In region V it may be present in small amounts when 3-carene is absent.

Next to terpinolene, β -phellandrene is the most common and abundant minor component. It is found in all regions and in most trees, but in trees in region IV and V it is frequently absent. It averages less than 1 percent but ranges to over 2 percent in some trees; it does not seem to be associated with other components. It is usually absent in trees high in α -pinene. Exceptions to its classification as a minor component were found in three trees in plot 44 near Lake Arrowhead in region I; percentages in those three trees were 10, 13, and 16 percent (table 15). This plot grows in a mixture with Coulter pine,

and these percentages are about what might be expected of an F_1 hybrid between Coulter pine and ponderosa pine. This is the first recording of a possible F_1 hybrid of these species. Attempts to produce this hybrid by controlled pollination have failed (Critchfield 1966).

Camphene is fairly common but not abundant. It is most abundant in region V, averaging about 0.7 percent. It decreases to about 0.4 percent in region IV and to about 0.1 percent in the other regions, where it frequently may be missing from a tree. The exceptions to the minor classification were found in plots 26 and 27, where camphene was 10 and 12 percent in two trees (table 15). Whether these high percentages are the result of some unusual accidents and nonheritable occurrences, or whether they are valid and heritable is yet to be determined.

The minor component γ -terpinene is never more than 0.3 percent, and is usually only a trace, averaging less than 0.1 percent. It occurs quite commonly and may be associated with 3-carene, but amounts are so small and elution so late that good quantitative associations cannot be made.

Thujene is almost always found in trace amounts, but infrequently it appears in measurable quantities, though even less than 0.5 percent. In the chromatogram, it is detected as a shoulder on the trailing edge of the α -pinene peak, so it may be difficult to detect where α -pinene is high. It is found most commonly in region III, where the amount of α -pinene is lowest.

Heptane, a 7-carbon hydrocarbon, was detected in trace amounts in very few samples. Since it elutes at the trailing edge of pentane, the solvent for all samples, slight amounts could be masked by the solvent peak.

Sesquiterpenes

The sesquiterpenes in some ponderosa pine xylem resin samples were analyzed by the chromatographic technique. The results must be considered preliminary because qualitative and quantitative determinations have not been thoroughly verified. Analyses were made on the short column; the samples were those used for monoterpene analyses. In one series of analyses, the sample from the first tree in each plot was used to determine regional patterns. In the other series, all the samples in plots 36, 12, and 7 were analyzed to determine plot variation; these three plots represent regions II, IV, and V, respectively.

The analysis of the first sample in each plot showed that longifolene is found in nearly all samples in all regions and zones except region II, where it was found less frequently. Cadenene and/or albi-caulene are found in most samples in region II but only infrequently in other regions and zones. The percent sesquiterpene of the terpene portion of resin averaged about 5 percent for these samples; many samples had less than 1 percent; a few were between 6 and 10 percent.

The analysis of the three full plots showed intra-plot variation to be low; the intraplot variation in monoterpenes in these same plots was quite large. Most of the samples in plot 36, representing region II, had cadenene as the most abundant; many samples had a smaller amount of longifolene as well. The samples from plots 12 and 7, representing respectively regions IV and V, had only longifolene. The average, maximum, and minimum sesquiterpene content of the terpene portion of resin was, respectively, plot 36—6, 12, and 2 percent; plot 12—6, 10, and 1 percent; plot 7—2, 5, and 0 percent.

Nearly every sample had small amounts of one to three unknown compounds which eluted near the sesquiterpenes. These never made up more than 1 percent of the terpene portion of resin.

The results of the sesquiterpene analyses are in general agreement with Mirov (1961), who found both cadenene and longifolene in ponderosa pine xylem resin; the amount ranged from 0 to 10 percent. Cadenene was found in Sierra Nevada samples and longifolene in the Rocky Mountain samples.

Resin and Bark Color, Fascicle Size

Resin Color

The scheme of colorless, light amber, amber, dark amber, yellow, medium yellow, and dark yellow covers the range of colors found in ponderosa pine resin. There is no consistent or reliable association of color and monoterpene composition. There is some degree of regional relationship; resin from regions IV and V is typically colorless to light amber, whereas in regions I and II, it tends toward yellow and dark amber. Although one might be inclined to associate the colorless resin with high α -pinene, colorless resin is found in all plots and in trees with very little α -pinene.

Bark Color

No clear local or regional pattern of bark color could be found. The bark of young trees is black to grey and heavily furrowed; with age it becomes plated and the color gradually changes to shades of brick red, reddish grey, or reddish yellow.

Fascicle Size

Number of needles per fascicle has been used as a general characteristic for defining the three geographic varieties of ponderosa pine: var. *scopulorum* with two needles per fascicle; var. *ponderosa* with three, and var. *arizonica* with four and five. In a recent study, Haller (1965) shows that climate and site have an effect on needle number. In Haller's study, region V was characterized by trees with four and five needles per fascicle, and some with three. Pelouquin, in an intensive study based on individual tree examinations, made similar findings.

The following is a generalized summary of the number of needles per fascicle in the plots of the regions and zones:

<u>Region or zone</u>	<u>Needles per fascicle</u>
I, A, II, B	3
III (northwest)	3
III (north)	Most 3, some 2
III (northeast)	Most 2, some 3
III (southeast)	Most 3, some 2
C, IV	Most 3, some 2
D	3, 4, and 5
V	4 and 5

The lack of close association between number of needles and terpene composition is obvious. An exception may be that the region-V-type tree is associated with trees with four and five needles per fascicle. A specific check was made on 10 region-V-type trees located in region IV; none had four- or five-needle fascicles but had three- and two-needle fascicles characteristic of region IV.

Relationships With Bark Beetles

Any attempt to associate resin characteristics with the absence, presence, and abundance of bark beetles that attack ponderosa pine has an inherent weakness of trying to draw lines where variable dimensions exist and interact. Another problem is the lack of a fixed definition of a host. Is it the species in which the beetle can reproduce under any condition

of the host, under certain conditions, or under very restricted conditions? In tree-insect relationships, abundance of both may be more important than distribution and yet conventional taxonomic classification is generally confined to a treatment of distribution. Therefore, the following discussion must be expressed in generalities.

Resin Quantity

Most of those who have worked with bark beetles—particularly beetles found on pine—have assumed that the amount of resin that beetles encounter during attack contributes to the success or failure of the attack. This assumption is backed by some reasonable evidence (Mason 1966, Smith 1972, Wood 1962). The capacity of ponderosa pine to exude resin can be affected by a wide array of biological and edaphic factors, such as moisture, temperature, tree age, stand structure. Furthermore, the effect of quantity can be influenced by quality. If ponderosa pines with no flow of resin can be assumed to be highly susceptible to most bark beetles, it would appear that about 5 percent of the ponderosa pine in Western United States are quite vulnerable to these insects. If trees with only a slight flow of resin are quite susceptible, the numbers of susceptible trees could be as much as 10 to 15 percent. And, on the other side of the scale, as many as 20 to 30 percent of the trees may be relatively resistant because of high rate of resin flow. Though trees may shift from one category to another, these conditions may be somewhat close to the prevailing situation.

Resin Quality

The effect of resin quality can decisively influence the effect of quantity. Conversely, quantity can affect quality. The possibility that trees with a high percentage of limonene may be resistant to western pine beetle attack has been reviewed in the section on individual tree types. Other relationships will be discussed here.

Smith and others (1969) suggest that western pine beetle infestations have been more destructive in areas where percent composition of the major monoterpenes is evenly balanced than in areas where one component dominates the composition. Regions I and II and zones A and B, where the western pine beetle historically has been most abundant and destructive, have the most balanced composition. And they have the greatest amount of β -pinene—the least toxic major monoterpene to west-

ern pine beetle. Furthermore, they have the highest percent of myrcene, the terpene that synergizes the attractive pheromones produced by western pine beetles. Thus, the greatest activity by the beetle fits certain monoterpene patterns: lack of high percentage of any one component, highest percentage of the least toxic monoterpene, and presence of the highest percentage of myrcene.

The genus *Dendroctonus* is closely but not exclusively associated with ponderosa pine throughout its range. Not all individual species of the genus are found throughout the tree's range in Western United States, but some species go beyond this range. Perhaps some property of the tree could contribute to the cause of why some beetle species do not develop throughout the tree's range.

The classification of *Dendroctonus* has had a recent major revision (Wood 1963) after the original classification of Hopkins (1911) had been long used with little change. Wood eliminated several species by combining them with others.

The seven species of *Dendroctonus* listed by Wood (1963) and Hopkins (1911) as occurring on ponderosa pine are listed below. The additional species listed by Hopkins which were placed under some of the seven by Wood, are given in parentheses after the species which now includes them.

- D. brevicomis* (*barberi*)
- D. arizonicus*
- D. frontalis* (*mexicanus*)
- D. approximatus*
- D. adjunctus* (*convexifrons*)
- D. ponderosae* (*monticolae*)
- D. valens*

In both classifications of *Dendroctonus*, the distributional limits of several species fall near the line separating a region and zone of this study. In Hopkins' classification, the northern limit of *D. barberi*, *D. convexifrons*, *D. arizonicus* and *D. approximatus* generally falls within zone C. In Wood's classification, the northern limit of *D. adjunctus* lies near the boundary between Region IV and Zone C; that of *D. approximatus*, between Region III and Zone C. Thus the insect complex and the resin composition of the tree make a decided shift at about these same lines. Whether this association between insect and tree is causal or coincidental has yet to be determined. A

valid test of a natural classification of *Dendroctonus* might be the relative tolerance of bark beetles to the various terpenes and the synergistic action of terpenes to bark beetle pheromones.

Other distributional relationships at first glance do not support the basic tenet of resin quality. Wood (1963) shows the range of western pine beetle to extend generally throughout the range of ponderosa pine in Western United States; but the beetle is rarely, if ever, found in the northeastern portion of Region III (Cascade Northern). On the basis of Hopkins' (1911) original classification, the distributional relationships hold a bit more firmly; *D. brevicomis* is fairly well limited to Regions I and II, and Zones A and B, and to the southwestern edge of Region III; *D. barberi* is found in Zones C and D and in Regions IV and V.

Region I has the highest average limonene, but *D. brevicomis* is fairly common—somewhat contrary to what might be expected. At least three explanations, singly or in combination could account for this condition. First, Region I also has the highest average of β -pinene, which is the least toxic terpene to *D. brevicomis*. Second, many individual trees do not have high limonene. Third, quantity influences the effect of quality.

Abundance of Resin Types

The abundance of individual types of resin composition in an area may be more important than the average composition of trees. Only a few types of trees have been exposed to regulated forced attack by the western pine beetle. Trees in which attacks have been easiest and most successful, however, resemble the two most abundant monoterpene types of ponderosa pine (Smith 1969). Possibly, the beetle subsists and thrives in these two types of trees, but if beetle population increases or if resin production is lowered, the beetle could be successful in attacking other types as well. Individual trees with a somewhat balanced percentage of three to five monoterpene components are found throughout the range of the tree, except in Region V. Such trees, though their abundance varies greatly, might also be more suitable hosts than trees that have only one or two components comprising a large percent of the monoterpenes.

CONCLUSIONS

This study of the xylem resin in ponderosa pine defines five regional and four transitional types of monoterpene composition. Such a classification supports the proposal that ponderosa pine should be treated as a complex species, with several varieties, ecotypes, or forms. The basis for this classification is that the average monoterpene composition of the resin of trees in plots within regions is similar, while in plots within zones, it is dissimilar. All regions and zones are significantly different from each other. Since this classification agrees only partially with others of ponderosa pine which are based on morphology, growth performance, or climate, resin composition appears to be generally independent of these other characteristics.

The percent of the terpene portion of the whole resin has a narrow range—regardless of resin source or monoterpene composition; therefore, it may be that ponderosa pine synthesizes a relatively constant amount of a terpene precursor. This characteristic may be common to all or most pine xylem resin systems.

In the trees studied, the monoterpene composition remained generally constant within a tree, but varied among trees and among test sites. The regional and local variation in composition can be explained on the basis of a large number of composition types; these types are derived from a hypothetical system which combines a two-allele gene system for each monoterpene with a nonequal intergene competition for the fixed amount of the terpene precursor. The difference in frequency of the monoterpene types of trees establishes the regional or transitional characteristics of monoterpenes in ponderosa pine. However, since many types are found in several regions or zones, the actual field situation must be consid-

ered dynamic. The transition zones are of particular interest since the incidence and frequency of monoterpene composition types changes over short distances.

No consistent association was found between monoterpene composition and rate of resin flow, sesquiterpenes, resin color, bark color, and needles per fascicle. This lack of association suggests that monoterpene composition is not closely linked with these other characteristics, and probably is under strong gene control.

Findings in this study have application to pest management strategy—particularly to research on the control of the western pine beetle and other bark beetles (*Dendroctonus* spp.). From 5 to 10 percent of the trees sampled may be unable to resist bark beetle attacks because they have little or no resin flow. An equally large or larger percentage may be conditionally resistant because they have copious resin flow. A small percentage may be inherently resistant because they have a relatively large amount of limonene.

The distribution limits of certain bark beetles may be associated with the regional and transitional limits of average monoterpene composition. The western pine beetle appears to be particularly destructive in areas where the concentration of the major monoterpenes is more equally balanced than elsewhere and where myrcene is most abundant. Myrcene synergizes the attractant pheromones of the beetle.

The knowledge of resin composition types, mode of inheritance of resin properties, and the effects of resin on beetle activity could be used in forest management practices and in the selection and breeding of ponderosa pines which are relatively resistant to western pine beetle.

SUMMARY

The xylem resin of more than 5,700 ponderosa pine trees (*Pinus ponderosa* Laws.) in Western United States was analyzed in a 9-year study; 5,165 of the trees were in 68 plots set up to represent the range of the species; the remainder were used for studies of sampling and plot size.

The amount of resin that flowed varied widely. Some trees yielded as much as 5 cm³ of resin in 2 to 4 hours, while nearby trees yielded none during the same period. Resin flowed from 95 percent of the trees during a tapping period of 8 to 12 hours. Weather and site conditions for tapping varied widely among regions and plots.

In trees more than 30 years old, no major effect on monoterpene composition could be attributed to within-tree position of sampling, time of sampling, or tree site. The monoterpene portion of whole resin averaged about 24 percent throughout the range of the tree. Two-thirds of the samples had portions ranging from 22 to 26 percent. No relation was found between monoterpene composition and percent terpene.

The quantity of the five major components in the monoterpene portion of individual trees varied widely over the range of the tree: α -pinene, 1 to 99 percent; β -pinene, 0 to 72 percent; 3-carene, 0 to 88 percent; myrcene, 1 to 51 percent; limonene, 0 to 70 percent. These five components make up more than 95 percent of monoterpenes. The distribution curves of the percentages of each component have regional characteristics.

The range of ponderosa pine can be conveniently divided into five regions and four transition zones on the basis of plot averages for the five major monoterpene components. Average plot compositions of these components were similar within regions, dissimilar within zones. All regions and zones were significantly different from each other in four or more components. This classification agrees only partially with others of ponderosa pine, which are based largely on morphology, growth performance, and climate.

A working genetic hypothesis, extrapolated from

the study and without corroboration from controlled breeding tests, is proposed. Each monoterpene has two alleles at a single locus with a value of 0 or 1 for the synthesis of that monoterpene. The pairs of alleles for each monoterpene are not equally competitive for the monoterpene precursor. A relatively constant amount of the precursor, as indicated by the fairly constant monoterpene portion of resin, is available to this genetic mechanism. The combinations of three levels of five intercompetitive components could explain the great variation and range in composition observed.

One hundred fifteen different types of monoterpene composition were found in applying the genetic hypothesis to the data on 4,894 trees. Thirty percent of the trees fall into two types, 95 percent into 54 types. Though certain types of composition characterize each region, these same types are found in other regions but less frequently. One type is found in all but one region and zone; its average composition is 6 percent α -pinene, 21 percent β -pinene, 51 percent 3-carene, 10 percent myrcene, 9 percent limonene.

One of the 115 composition types is high in limonene. It may prove to be resistant to attacks by the western pine beetle (*Dendroctonus brevicomis* Lec.), a major forest pest in Western United States. Its composition is about 20 percent α -pinene, 0 percent β -pinene, 0 percent 3-carene, 20 percent myrcene, and 60 percent limonene. Five trees of this type were found in the study.

No association could be found between resin color and monoterpene composition. Nor could any association be established between composition and needles per fascicle.

The distribution limits and severity of attack of certain bark beetles can be associated, in some degree, with monoterpene composition. The western pine beetle has been the most damaging where the five major components occur in the most evenly balanced percentages and where myrcene, which synergizes the attractant pheromones of beetles, is most abundant.

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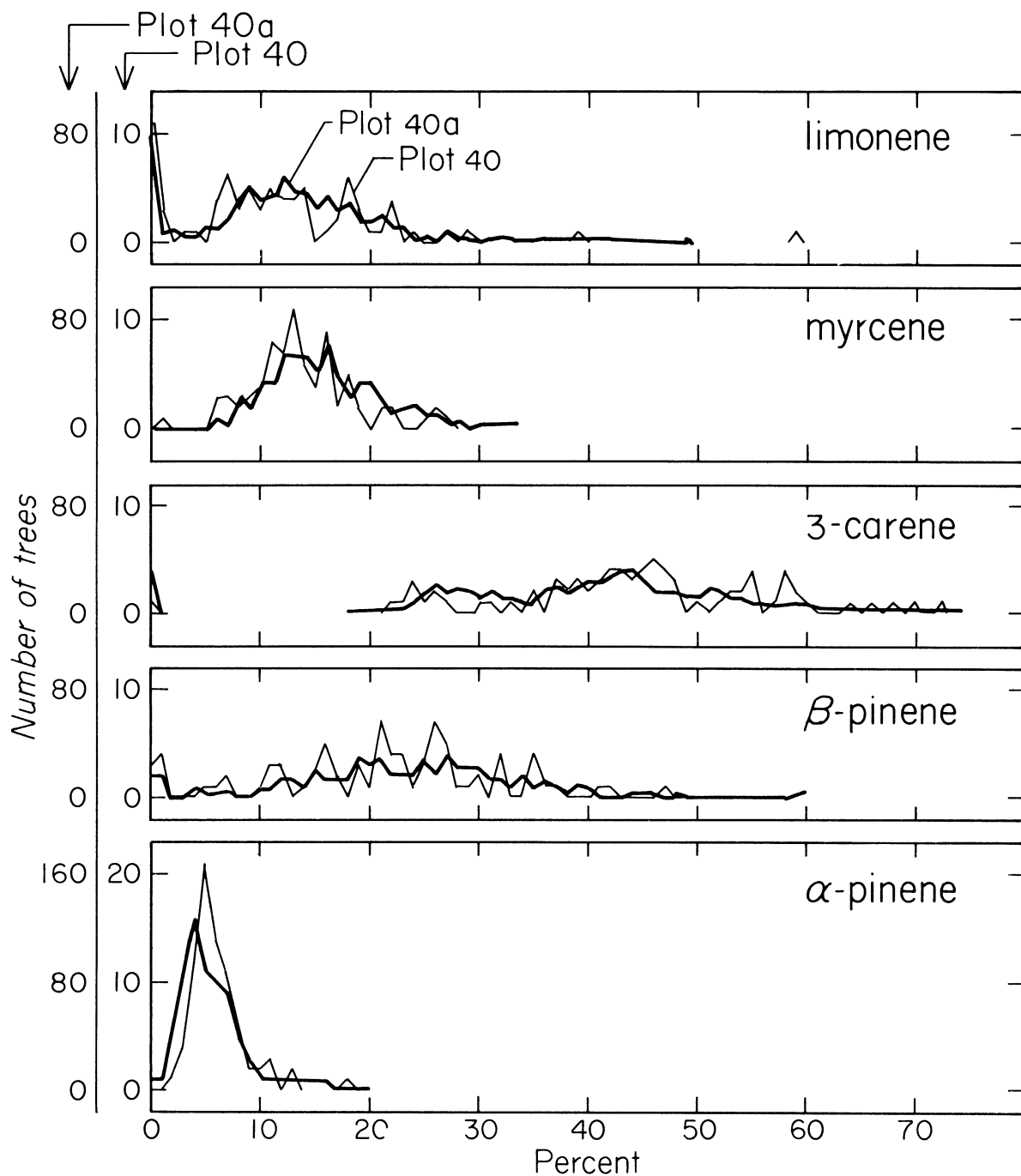
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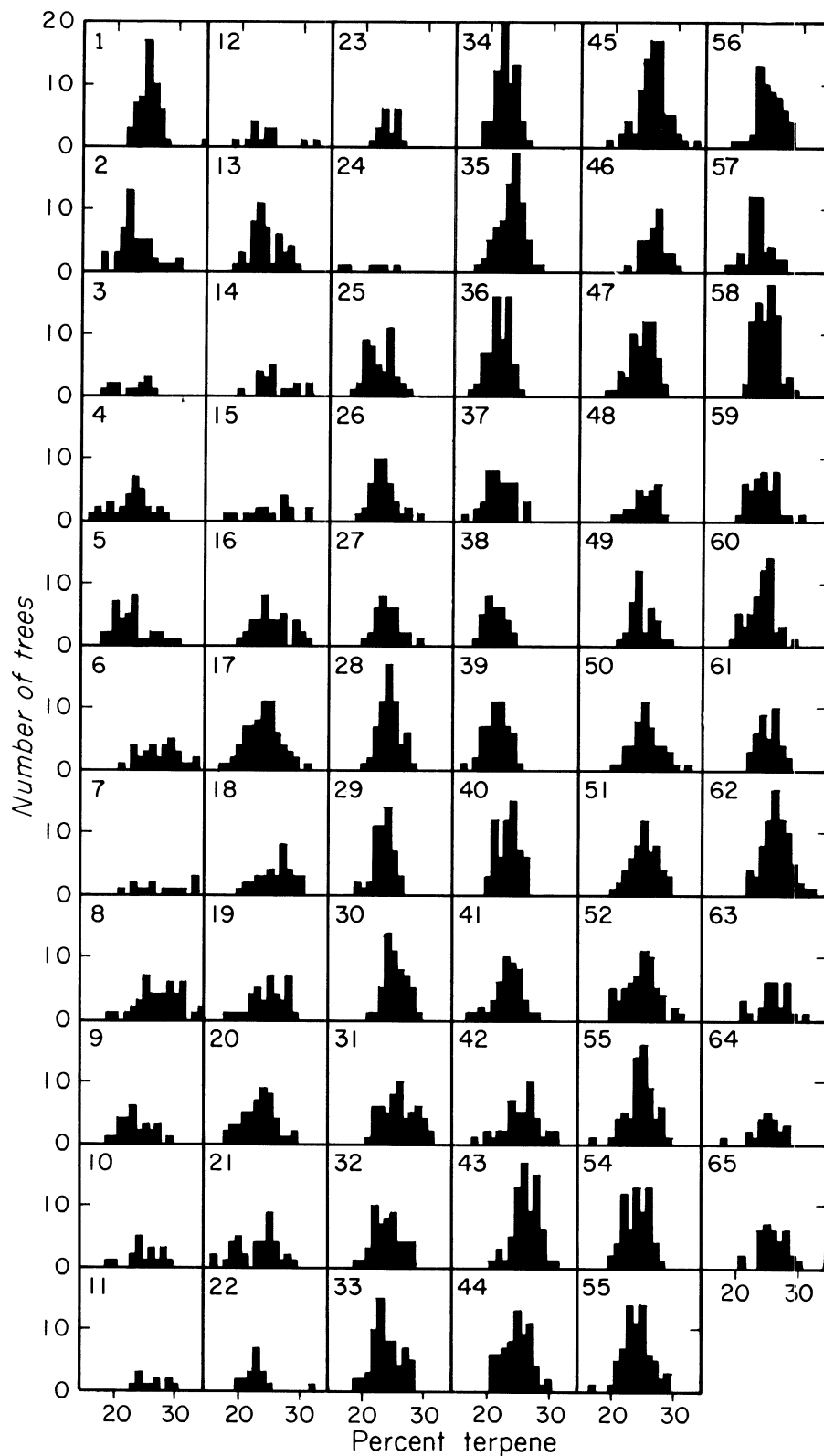
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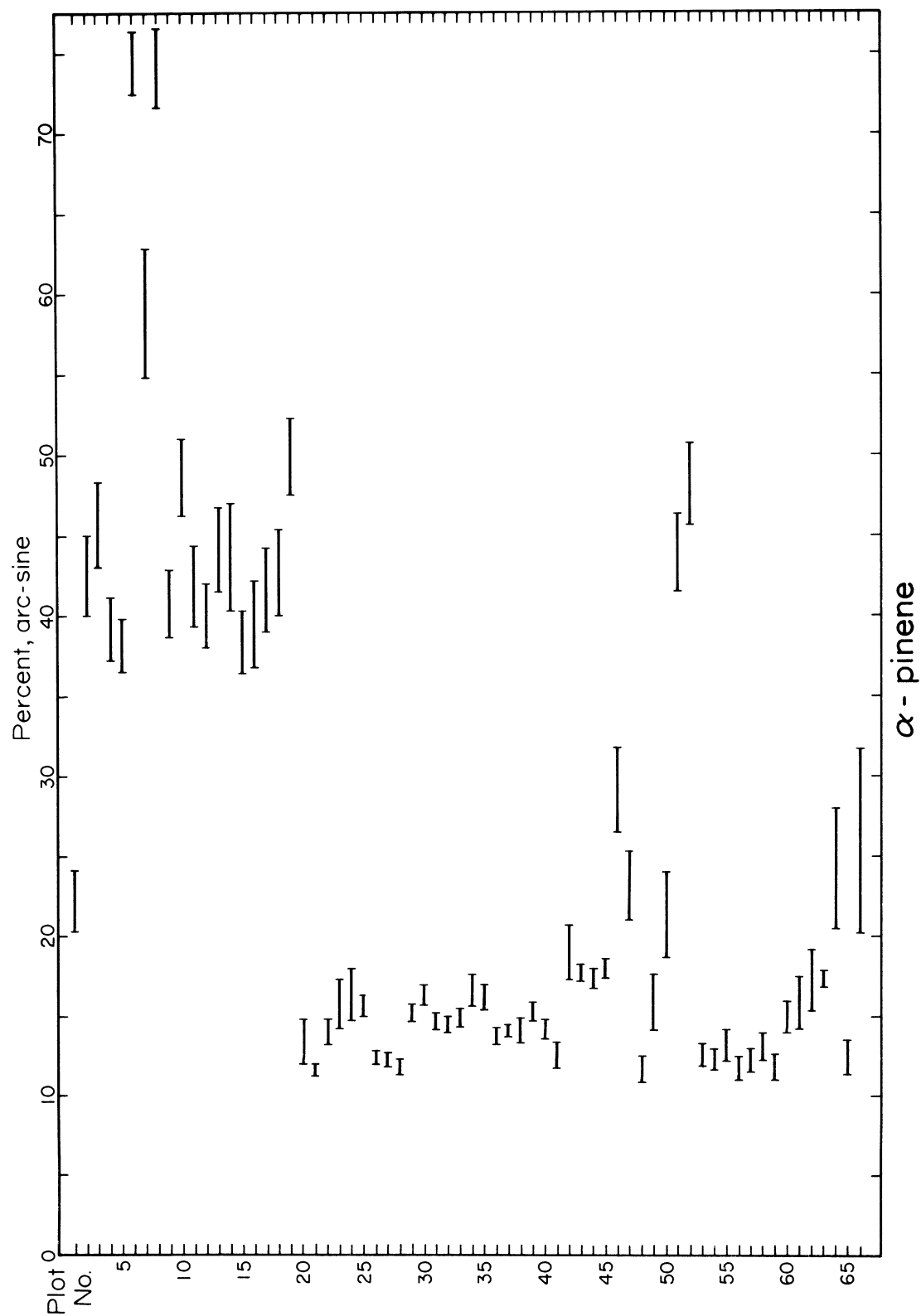
APPENDICES



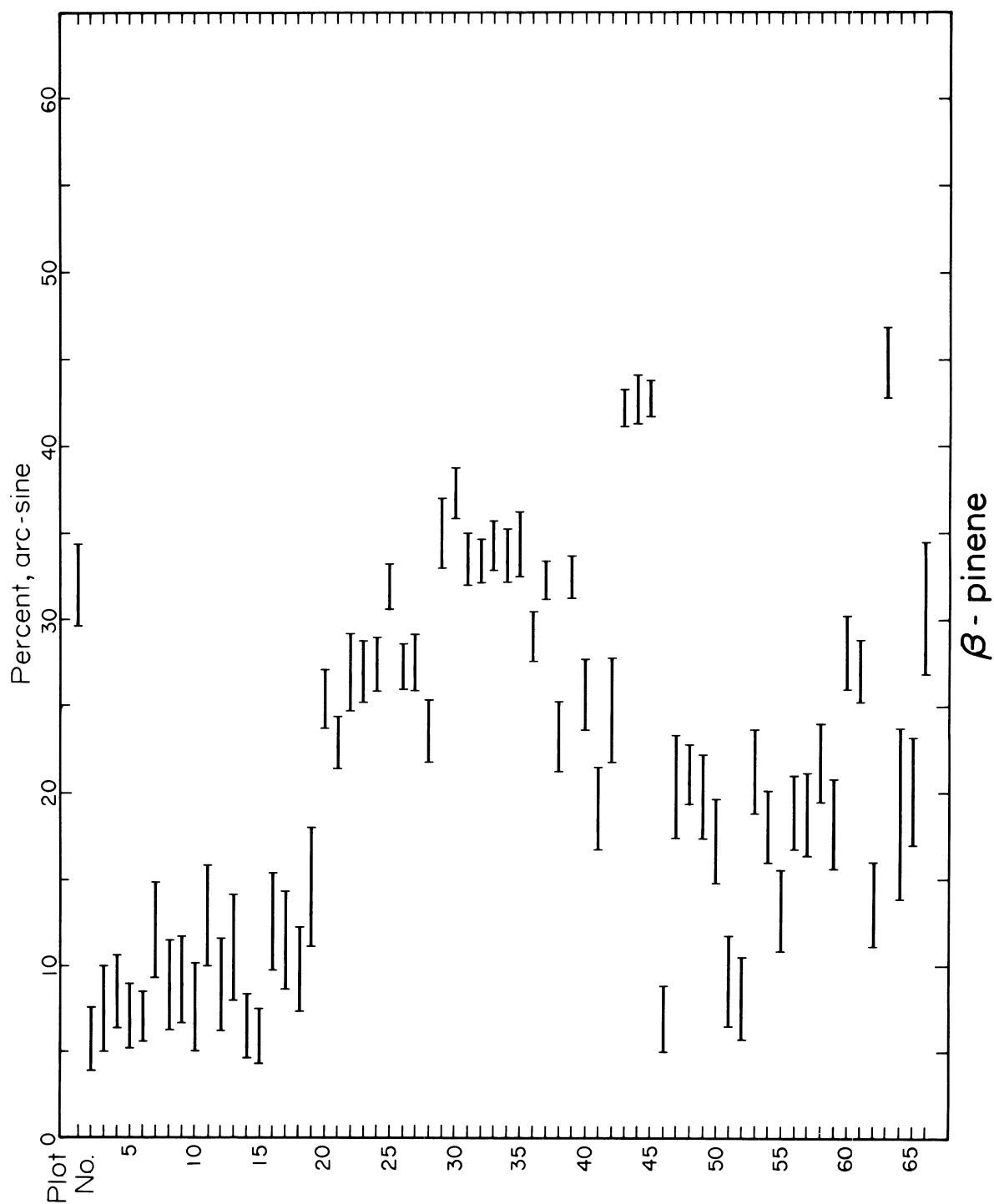
Appendix I.—Frequency distributions of five major components— α -pinene, β -pinene, 3-carene, myrcene, limonene—are given for 80 trees in plot 40 and for 624 trees in plot 40a Modoc National Forest, Calif.



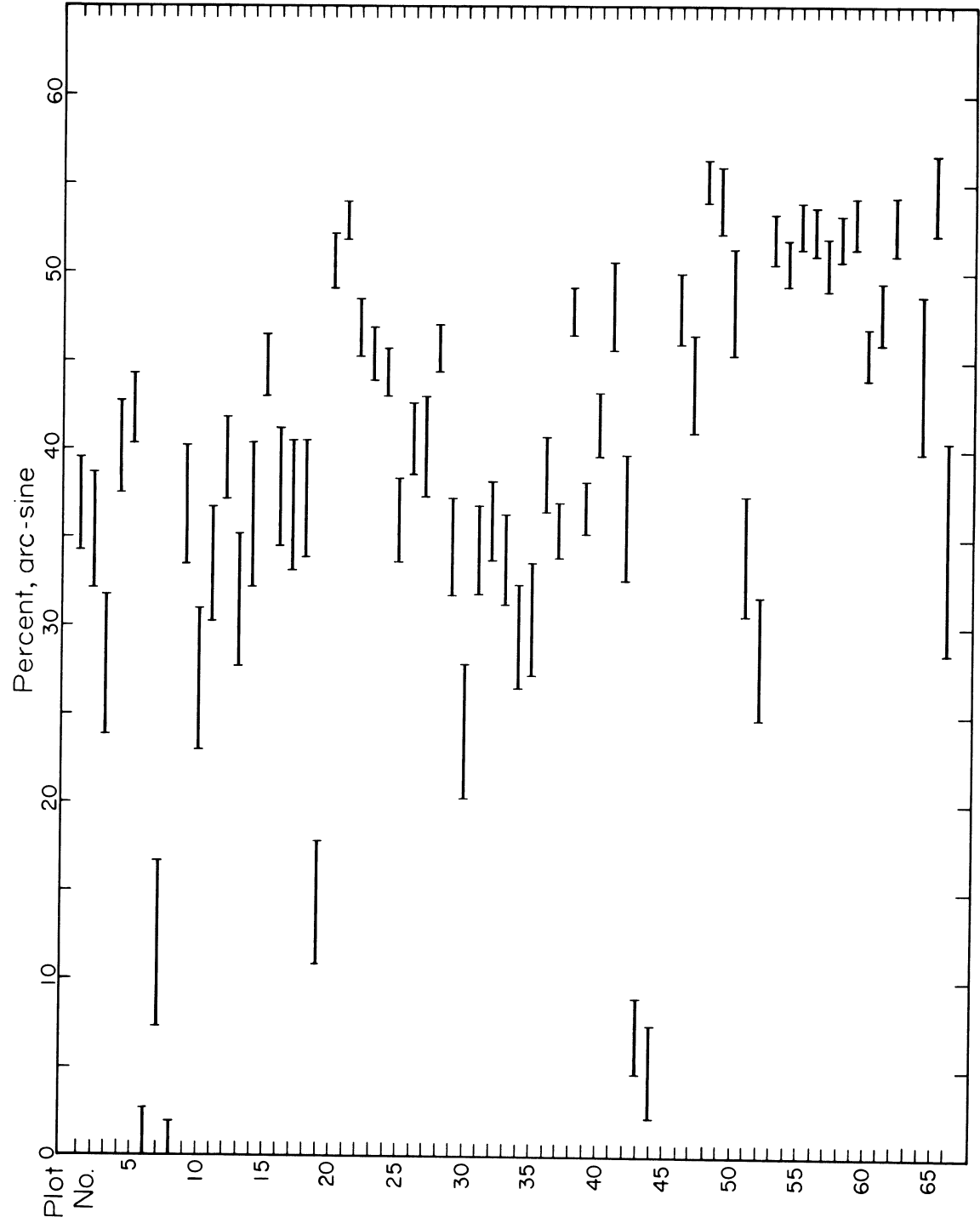
Appendix II.—Percent of terpene in the whole resin of ponderosa pine varied only slightly among the 65 plots from which samples were taken for the study.



Appendix III.—Confidence bars at 95 percent level for each of the five major monoterpene components— α -pinene, β -pinene, 3-carene, myrcene, and limonene—of ponderosa pine xylem resin in 66 plots; percents have been converted to arc-sine values.



Appendix III.—Continued



3 - carene

Appendix III.—Continued

